JOURNAL

OF THE

AMERICAN SOCIETY

OF

SUGAR BEET TECHNOLOGISTS

VOL. IX, NO. 2 JULY, 1956

EXECUTIVE COMMITTEE AND ADVISORY COUNCIL

President ——— Austin Armer
Spreckels Sugar Company, Woodland, California

Vice President ——— Frank Rawlings
Amalgamated Sugar Company, Ogden, Utah

Secretary-Treasurer ——— James H. Fischer
Beet Sugar Development Foundation, Fort Collins, Colorado

Advisory Council

West Coast Area:	C. W. Bennett G. D. Manuel
Intermountain Area:	Bion Tolman Harold E. Ellison
Eastern Slope Area:	E. H. Hungerford H. P. H. Johnson C. M. Nicholson
Eastern U. S. Area:	Grant Nichol M. G. Frakes
Eastern Canadian Area:	C. A. Neil
Western Canadian Area:	Wesley Smith
At Large:	Lyman Andrews Vernal Jensen

Manuscripts submitted for publication and communications pertaining to editorial matters should be sent to James H. Fischer, Secretary-Treasurer, American Society of Sugar Beet Technologists, P. O. Box 538, Fort Collins, Colorado.

Each manuscript received for publication will be appraised for its technical and historical value by an Editorial Board. The Editorial Board shall have final authority regarding publication of manuscripts. The Journal of the American Society of Sugar Beet Technologists shall contain papers presented at General and Regional meetings and articles of immediate interest prepared specifically for this periodical.

Material submitted to the Editorial Board for publication becomes the property of the American Society of Sugar Beet Technologists. The Editorial Board will have jurisdiction over the release of papers and abstracts for use other than in the Journal.

JOURNAL
of the
American Society of Sugar Beet Technologists

Volume IX
Number 2
July, 1956

Published quarterly by

The American Society of Sugar Beet Technologists
Office of the Secretary
P. O. Box 538
Fort Collins, Colorado, U. S. A.

Subscription prices:

$4.50 per year, domestic
$5.00 per year, foreign
$1.25 per copy, domestic
$1.40 per copy, foreign

Made in the United States of America

TABLE OF CONTENTS

	Author	Page
The influence of antecedent climates upon the subsequent growth and development of the sugar beet plant	*Albert Ulrich*	97
Nitrogen constituents associated with reduction of sucrose percentage and purity of sugar beets	*J. L. Haddock* *D. C. Linton* *R. L. Hurst*	110
Silver Engineering's new grab-roll beet screen	*Clarence Steele*	118
Treatment of sugar beet seed with systemic insecticides for control of the beet leafhopper	*Orin A. Hills* *F. H. Harries* *A. C. Valcarce*	124
Isolation and properties of sugar beet araban	*Alan E. Goodban* *Harry S. Owens*	129
Inheritance of raffinose production in the sugar beet	*R. R. Wood* *R. K. Oldemeyer* *H. L. Bush*	133
Effects of sugar beet root diffusates and extracts, and other substances, on the hatching of eggs from the cysts of the sugar beet nematode, *Heterodera schachtii* Schmidt.	*Gerald Thorne*	139
Determination of saponin in granulated sugar: method of H. G. Walker adapted to routine control	*Robert R. West* *Robert S. Gaddie*	146
Materials and methods used in producing commercial male-sterile hybrids	*Bion Tolman* *Ronald Johnson*	153
Drying temperatures and storage problems of sugar beet seeds	*Carl W. Hall*	161
Experience with auto-jet filters	*George W. Cossairt* *Louis E. Purmort*	167

Breeding of sugar beets with reference to sodium, sucrose, and raffinose content. *R. E. Finkner, H. M. Bauserman* 170

Resistance of inbred varieties of sugar beets to aphanomyces, rhizoctonia, and fusarium root rots. *M. M. Afansiev* 178

Some effects of excess water application on utilization of applied nitrogen by sugar beets. *J. S. Robins, C. E. Nelson, C. E. Domingo* 180

Studies of the control of saponin concentration in refined beet sugar. *Robert S. Gaddie* 189

The Influence of Antecedent Climates Upon the Subsequent Growth and Development of the Sugar Beet Plant

ALBERT ULRICH[1]

The main factors that are known to influence the growth and development of the sugar beet plant are its genetic composition, climatic environment, nutritional status, and the prevalence of diseases and pests. Of these, the effect of climate upon the beet plant has been the least studied. This deficiency in our knowledge of the specific effects of climate upon the growth of the sugar beet plant has evolved from a lack of suitable facilities for growing plants in simulated climates. Most of our information concerning sugar beets has been obtained through chance observations under field conditions, in which good growth was correlated with favorable temperatures and light conditions, and by relating poor growth with frosts and excessively high temperatures, cloudy weather, high wind velocities or low rainfall.

When the Phytotron at the California Institute of Technology, Pasadena, California, was completed in 1949 under the direction of F. W. Went (5)[2], experiments pertaining to the effects of light, day length, night and day temperatures became readily possible. The results of these experiments have been reported earlier (3, 4). Since then, other studies have been completed in the Pasadena facilities. One of these has been a comparison of the effects of climatic sequences upon the beet plant, such as a "cold spring" followed by a "mild" or a "hot summer," as contrasted with a "mild" or even "hot spring" followed by a "mild" or "cold summer." The results of these and other climatic sequences, as they affect top and beet root growth and the sucrose content of the beet root, are reported in this paper.

Methods and Materials

On May 14, 1952, seeds of the sugar beet variety U.S. 22/3, treated with Phygon XL (1 gram per 100 grams of seed), were planted at the rate of 10 seedballs per pot into vermiculite No. 2 at a depth of ¾ inch. A modified Hoagland nutrient solution (2) was added immediately after planting and at daily intervals thereafter for the duration of the experiment. The pots used were standard metal containers 10 inches in diameter and 12 inches deep having a sanitary lacquer lining on the inside and painted with aluminum on the outside. Drainage was provided by means of four small holes at the bottom of the containers. All 108 pots were filled by adding, in rotation, small amounts of vermiculite. When the pots were filled, they were watered, tamped lightly by dropping the pots vertically on the floor, refilled and tamped again until no further settling took place.

The pots were distributed to a total of 27 trucks, with 4 pots per truck. The trucks were divided into 3 groups with 9 trucks per group. Each group was placed prior to planting into its initial climatic condition. These conditions were:

---

[1] Plant Physiologist, University of California, Berkeley 4, California.
[2] Numbers in parentheses refer to literature cited.

	"Day temperature" 8:00 a.m. to 4:00 p.m.	"Night temperature" 4:00 p.m. to 8:00 a.m.
Cold	17° C. (63° F.)	12° C. (54° F.)
Mild	23° C. (73° F.)	17° C. (63° F.)
Hot	30° C. (86° F.)	22° C. (72° F.)

All plants were subjected to natural daylight and natural day lengths under greenhouse conditions of the Phytotron, California Institute of Technology, Pasadena, California.

The seedlings emerged on May 19, in the mild and hot climates, and on the following day in the cold climate. On May 29 the plants in the mild and hot climates were in the 2-3 leaf stage. These were thinned to one seedling per seedball. Two weeks later, on June 12, the mild and hot climate plants (6-7 leaf stage) were thinned to four seedlings per pot. On this date the plants in the cold climate were in the 4-leaf stage and were thinned to one seedling per seedball. The final thinning to two plants per pot for the mild and hot climate plants (12-leaf stage) was done on June 24. The cold climate plants (9-leaf stage) were thinned on this day to four plants per pot and on the following day to two plants per pot.

In order to equalize the effects of position on plant growth within a climate, the trucks were re-randomized at weekly intervals. Also, at weekly intervals, old leaf and new leaf counts were made. The plants were allowed to grow in their initial climate for a period of 56 days, May 14 to July 9, 1952. On July 9, the plants were placed into their final climatic conditions. Of the 9 trucks originally in a given initial climate, 3 trucks were allowed to remain in the initial climate and the other 6 trucks were divided into 2 groups of 3 trucks each, and then placed into the two remaining climates. Thus, in any one final climate, there were 3 trucks which were there originally and 3 trucks from each of the other two initial climates.

The plants were subjected to the final climatic condition until September 22, 1952 (75 days), at which time the plants were harvested. During the harvest, each plant was separated as follows: (a) Blades of recently matured leaves; (b) other living blades more than one inch wide; (c) petioles of recently matured leaves; (d) unclassified material (petioles, immature leaves and tip of stem); and (e) root. The tops were cut off at the point of the oldest remaining living leaf. The beet roots were trimmed slightly to remove the remaining dead petiole tissue.

Sucrose samples consisting of 26.0 grams of beet pulp were taken from the combined storage roots for each pot and placed in glass jars, then immediately frozen with dry ice. Sucrose was determined in duplicate, using a saccharimeter and the hot extraction method of Browne and Zerban (1).

The temperatures of the three rooms were recorded automatically by a 16 point Brown temperature recorder.

Results

Beet root weights: The beet root weights were influenced greatly by the cold, mild and hot climates of the initial and final growth periods (Tables 1 and 2). The effects of the initial climate were noticeable even

Table 1.—Summary of F-values and Error Variances of Beet Plants in Relation to Initial (I) and Final (F) Climates.

Source of Variation	D.F.	Required F-values		Observed F-values Relative to Error *a*								
				Beet Roots			Tops		Living leaves			
				Fresh	Sucrose		Fresh	Dry	Old leaves dry wt.		Blades fresh	Blades as % of total tops
		5%	1%	gm	%	gm	gm	gm	gm	Number	gm	
Treatments	8	2.51	3.71	6.73	40.53	7.97	17.83	21.42	7.70	13.13	20.25	10.65
Climate (I)	(2)	3.55	6.01	7.26	1.41	7.86	13.60	7.20	2.57*	1.87	3.84	10.73
Climate (F)	(2)	3.55	6.01	19.51	158.60	22.92	52.74	71.84	27.60	49.54	75.41	22.70
I x F	(4)	2.93	4.58	0.08	1.06	0.25	2.50	3.32	0.32	0.57	0.87	4.58
Error *a*[1]	18	.....	.....	12,991	0.866	127.29	18,110	172.29	58.95	99	1601	25.75
Error *b*[2]	81	2.00	2.71	31,786	0.568	333.84	31,820	324.39	136.85	95	3889	17.95

[1] Error *a* is the variance between trucks of the same treatment.

[2] Error *b* is the variance between pots within a truck. Error *b* significance is relative to error *a*.

Table 2.—Beet Root Weights in Grams per Pot.[1]

Initial Climate	Final Climate July 9-Sept. 22 (75 Days)			Initial Climate Mean
May 14-July 9 (56 Days)	Cold 63-54° F.	Mild 73-63° F.	Hot 86-72° F.	
Cold	300	432	274	335
Mild	395	522	388	435
Hot	356	510	347	404
Final climate mean	350	488	337	

[1] Significant differences within climates are 98 and 134, and between climates 56 and 77, at the 5% and 1% levels, respectively.

though the initial growth period of 56 days was followed by a final period of 75 days in another climate. The observed F-value for the final 75-day period, as expected, was much larger than the F-value for the initial period of 56 days (Table 1). The low F-value observed for the interaction of the two growth periods implies that the climatic effects were strictly additive and were not dependent upon the climate that preceded or followed a given climate. Whether there would have been a significant interaction had the temperatures been lower so as to induce flowering, or higher initially or finally so as to alter still more the metabolism of the plant, is a point worthy of further study.

The effects of the initial climate on storage root growth may be observed from the storage root weights of the plants initially in the cold, mild or hot climate, regardless of the final climate (Table 2). The plants initially in the cold climate averaged 335 grams and this is significantly less than the 435 and 404 grams for the plants originally in the mild and hot climates. The average root weights for the final climates, regardless of initial climate, were 350, 488, and 337 grams per pot for the cold, mild, and hot climates, respectively (Table 2). Since these weights differ much more than those for the initial climates, this accounts for the F-value being larger for the final than for the initial climate (Table 1).

The results presented in Table 2 also indicate that the cold climate plants made very poor root growth when the plants were retained in the cold climate (300 grams) or when comparable plants were transferred to a hot climate (274 grams). When the cold climate plants were moved to the mild climate, a distinct improvement in growth took place (432 grams). The best root growth, however, was made by the plants continuously in the mild climate. These roots weighed 522 grams per pot. When comparable plants of the mild climate were moved to the cold or hot climates, the root weights were 395 grams and 388 grams per pot, respectively. Plants continuously in the hot climate had roots that weighed 347 grams, but when moved to the mild or cold climates these weighed 510 and 356 grams per pot, respectively. Thus, root growth was favored whenever the plants were kept continuously or even partly in a mild climate.

Sucrose concentration: The sucrose concentrations of the roots were not influenced by the initial climate under which the plants had been grown. This is shown by the lack of significance of the initial climate F-values, Table 1, and by the uniformity of the initial climate means presented in Table 3. There was, however, a tremendous effect of the final climate upon

Table 3.—Percent Sucrose[1]

Initial Climate	Final Climate July 9-Sept. 22 (75 Days)			Initial Climate Mean
May 14-July 9 (56 Days)	Cold 63-54° F.	Mild 73-63° F.	Hot 86-72° F.	
Cold	11.45	8.72	7.49	9.22
Mild	12.00	8.70	7.98	9.56
Hot	11.40	9.08	8.07	9.52
Final climate mean	11.62	8.84	7.85	

[1] Significant differences within climates are 0.80 and 1.09 and between climates 0.46 and 0.63 at the 5% and 1% levels, respectively.

the sucrose concentration of the beet roots. The plants in the cold climate had an average sucrose concentration of 11.62%, in the mild climate 8.84%, and in the hot climate 7.85%. These findings are in accord with those reported earlier, that is, low temperatures are conducive to sucrose accumulation and high temperatures tend to depress the sucrose concentration of the storage root (3, 4). Again the interaction of initial and final climate was not significant (Table 1).

The finding that the sucrose concentration of the root is independent of the antecedent climate has a practical implication, especially if the time required to effect a change during the final growth period is rather brief. Thus, if it is found that it takes only two weeks to change the sucrose concentration of beets from 7.85% in a hot climate to that of 11.62% in a cold climate, then the proper climatic measurements made prior to harvest may offer a clue as to the trends that are to take place in the sucrose concentrations of the beets as the season progresses. An integrated value of the climatic measurements could then serve as a guide to the harvesting of sugar beets, assuming some leeway as to the time of harvesting the crop in a given district.

Sucrose stored: An inspection of the results presented in Tables 1 and 4 indicates rather clearly that the initial period of 56 days had a pronounced effect upon the sucrose stored in the root. The effect of the final period of 75 days was even greater than that of the initial growth period. The F-value for interaction was again not significant and this indicates that the initial and final climate effects were additive and not dependent upon each other. The largest amounts of sugar were stored in the plants that

Table 4.—Sucrose in Grams per Pot.[1]

Initial Climate	Final Climate July 9-Sept. 22 (75 Days)			Initial Climate Mean
May 14-July 9 (56 Days)	Cold 63-54° F.	Mild 73-63° F.	Hot 86-72° F.	
Cold	34.4	38.2	20.5	31.0
Mild	47.3	46.0	31.6	41.6
Hot	41.4	46.2	28.4	38.7
Final climate mean	41.0	43.5	26.8	

[1] Significant differences within the table at the 5% and 1% levels are equal to 9.7 and 13.3 and between climate means 5.6 and 7.7, respectively.

had been grown in the cold and mild climates during the final 75 day growth period (Table 4). The plants that ended their growth period in the hot climate were exceedingly low in sugar stored. This was the result of the combined effects of low sucrose concentrations (Table 3) and smaller beet root sizes (Table 2). The small amount of sugar stored in the plants kept continuously in the cold climate (34.4 grams per pot) was primarily a matter of slower root growth and not of lower sucrose concentration. Of special interest is the observation that the plants initially in the hot climate and then transferred either to a cold or intermediate climate were not materially altered in sugar stored (Table 4). Apparently early hot weather is not nearly so detrimental as hot weather late in the season.

Table 5.—Fresh Weight of Tops in Grams per Pot[1]

Initial Climate	Final Climate July 9-Sept. 22 (75 Days)			Initial Climate Mean
May 14-July 9 (56 Days)	Cold 63-54° F.	Mild 73-63° F.	Hot 86-72° F.	
Cold	752	656	356	588
Mild	594	642	329	522
Hot	456	531	284	424
Final climate mean	600	610	323	

[1] Significant differences within climates are 115 and 158, and between climates 67 and 91 at the 5% and 1% levels, respectively.

Fresh weight of tops: The results presented in Tables 1 and 5 indicate that there was not only a large effect of the final climate on top growth, but that the initial climate influenced the top weights greatly. The largest effect of the initial climate on top weight may be seen in the plants which were transferred from the hot to the cold climate. Here the top weights increased from 284 grams per pot to 456 grams, as compared to 752 grams per pot for plants continuously in the cold climate (Table 5). The largest effect of the final climate may be seen for the cold climate plants moved to a hot climate. These plants decreased in top weight from 752 grams to 356 grams per pot, a value which is only slightly better than the 284 grams per pot for plants continuously in the hot climate. During cold weather the tops remain green and the leaves tend to accumulate without dying, whereas hot weather occurring either early or late in the growing season is obviously detrimental to top growth.

Table 6.—Dry Weight of Tops in Grams per Pot.[1]

Initial Climate	Final Climate July 9-Sept. 22 (75 Days)			Initial Climate Mean
May 14-July 9 (56 Days)	Cold 63-54° F.	Mild 73-63° F.	Hot 86-72° F.	
Cold	78.4	51.5	29.4	53.1
Mild	65.6	52.9	28.6	49.0
Hot	51.1	46.9	26.7	41.6
Final climate mean	65.1	50.4	28.2	

[1] Significant differences within climates are 11.3 and 15.4, and between climates 6.5 and 8.9, at the 5% and 1% levels, respectively.

Dry weight of the tops: The dry weights of the tops (Table 6) are quite similar to the corresponding fresh weights (Table 5). The effects of both the initial and final climates are highly significant. The F-value for the interaction of the initial and final climate exceeds the 5% level of significance, instead of being somewhat short of significance as observed for the fresh weights of the tops (Table 1). A careful inspection of the dry weights of the tops (Table 6) indicates that this interaction has arisen primarily because the plants initially in the hot climate failed to recover fully when placed in the cold climate. The tops of these plants weighed only 51.1 grams in comparison to the 78.4 grams for the plants continuously in the cold climate. At the same time the plants initially in the cold climate, when placed in the hot climate, weighed only 29.4 grams. This compares with the 26.7 grams for the plants continuously in the hot climate. Thus, by moving the plants from the hot to the cold climate the tops nearly doubled in weight, whereas by moving the plants from the cold climate to the hot climate, the tops weighed only one-third of those in the cold climate. Apparently, the recovery of plants from the effects of a hot climate takes place more slowly than injury to tops when the plants are moved from a cold to a hot climate.

Table 7.—Fresh Weight of Blades in Grams per Pot.[1]

Initial Climate	Final Climate July 9-Sept. 22 (75 Days)			Initial Climate Mean
May 14-July 9 (56 Days)	Cold 63-54° F.	Mild 73-63° F.	Hot 86-72° F.	
Cold	222	189	105	172
Mild	230	177	97	168
Hot	188	159	95	147
Final climate mean	213	175	99	

[1] Significant differences within the table at the 5% and 1% levels are equal to 34.3 and 47.0 and between climate means, 19.8 and 27.1, respectively.

Fresh weight of blades: The fresh weight of the blades is also closely related to the fresh weight of the tops, except that the initial climate had a very much smaller effect upon the blades (Tables 1 and 7) than upon the fresh or dry weights of the tops (Tables 1 and 5). This indicates that, if one is seeking an attribute of the beet plant which is closely associated with climate, weighing leaf blades would be a better measure of the effect than weighing the entire tops of the plants.

Table 8.—Blades as Percent of Total Weight of Top (Fresh Basis).[1]

Initial Climate	Final Climate July 9-Sept. 22 (75 Days)			Initial Climate Mean
May 14-July 9 (56 Days)	Cold 63-54° F.	Mild 73-63° F.	Hot 86-72° F.	
Cold	30.0	29.1	29.4	29.5
Mild	38.6	27.7	29.4	31.9
Hot	41.4	30.0	33.7	35.0
Final climate mean	36.7	28.9	30.9	

[1] Significant differences within climates are 4.4 and 6.0, and between climates 2.5 and 3.4 at the 5% and 1% levels, respectively.

Blades as percent of total tops: The values for the fresh weight of the blades expressed as percent of the total fresh weight of the tops are presented in Tables 1 and 8. The F-values reported in Table 1 indicate that the interaction of the effects of initial and final climates is highly significant, as are the main effects of the initial and final climates. The main effects, however, may be the result of the interaction rather than due to the specific effects of climate upon this percentage. This may be seen in Table 8 where the largest increase in the percentage of blades to total tops took place in the plants in the cold climate from July 9 to September 22, 1952. These percentages increased gradually from 30.0 for the plants continuously in the cold climate to 38.6 and 41.4 for the plants initially in the mild and hot climates, respectively. All other percentages are approximately the same as for the plants continuously in the cold climate, namely, 30.0 percent. Other than this effect of cold climate on the blade to top ratio there seems to be no consistent pattern of this ratio to growth or to sucrose concentration.

Dry weight of old leaves: The F-values tabulated in Table 1 indicate that the dying of old leaves is more closely associated with the final climate than with the initial climate. Interesting too, is the fact that the highest weight of old leaves is to be found for the plants in a hot climate (Table 9), which is just the converse of that found for the fresh tops (Table 5), or for the dry tops exclusive of the old leaves (Table 6). The increase in old leaf formation is another indication that the sugar beet plant grows better in a mild climate, or even in a relatively cold climate, than in a hot climate.

Table 9.—Dry Weight of Old Leaves in Grams per Pot.[1]

Initial Climate	Final Climate July 9-Sept. 22 (75 Days)			Initial Climate Mean
May 14-July 9 (56 Days)	Cold 63-54° F.	Mild 73-63° F.	Hot 86-72° F.	
Cold	25.6	36.4	39.6	33.9
Mild	29.0	40.7	41.3	37.0
Hot	31.2	38.8	43.1	37.7
Final climate mean	28.6	38.6	41.3	

[1] Significant differences within climates are 6.6 and 9.0, and between climates 3.8 and 5.2, at the 5% and 1% levels, respectively.

Kind and number of leaves formed: The sugar beet plants that were grown in the cold, mild, and hot climates differed tremendously in outward appearance. Both the blade and petiole tissues of the leaves of plants in the cold climate were a dark green color. The blades were generally broad and rounded in shape and the petioles relatively short and thick in structure. In sharp contrast to this sturdy appearance of the leaves of the beet plants in the cold climate, the blades of the plants in the hot climate were pale green and the petioles a pale yellow color to almost white. The blades of these leaves were long and narrow with the outer edges of many curled upward. The corresponding petioles were long, brittle, and narrow in structure. Intermediate to the leaves in the cold and hot climates were those of the plants in the mild climate. The leaves of these plants were similar to those found in many sugar beet areas during midseason and were characterized as being exceedingly vigorous by all observers familiar with the culture of the sugar beet plant.

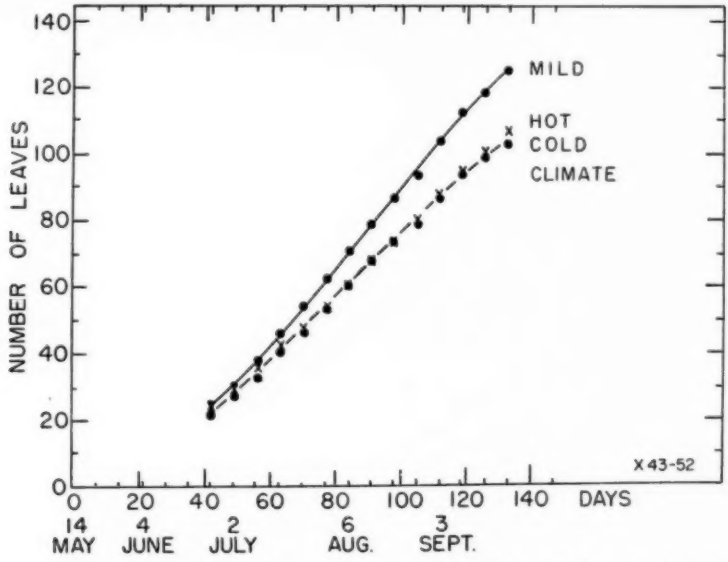


Figure 1.—Total number of leaves produced in relation to time by sugar beet plants continuously in a cold, mild (temperate) or hot climate. The leaf counts are on a pot basis of two plants per pot, using 12 pots per climate.

Table 10.—Number of Living Leaves per Pot at Harvest.[1]

Initial Climate	Final Climate July 9-Sept. 22 (75 Days)			Initial Climate Mean
May 14-July 9 (56 Days)	Cold 63-54° F.	Mild 73-63° F.	Hot 86-72° F.	
Cold	67.8	64.8	43.5	58.7
Mild	67.4	71.1	50.8	63.1
Hot	65.9	67.1	47.1	60.0
Final climate mean	67.1	67.6	47.1	

[1] Significant differences within climates are 8.5 and 11.7, and between climates 4.9 and 6.7 at the 5% and 1% levels, respectively.

Even though there were tremendous differences in the kind of leaf developed by the beet plants in the cold, mild, and hot climates, there was very little difference in the total number of leaves formed by the beet plants in these climates (Figure 1). There was also no effect of the initial climate upon the number of living leaves present at time of harvest (Table 1) nor was there any interaction of the initial and final climates on the number of living leaves for these plants. Even though the differences in the number of living leaves relative to the final climate were highly significant statistically, the differences in actual numbers were not large at the time of harvest (Table 10), nor during the growing periods of the plants in the different climates (Figures 2, 3, 4). A study of these figures reveals that the

number of living leaves in the cold climate (Figure 2) tends to increase gradually, whereas those in the mild climate (Figure 3) may or may not reach a plateau, depending most likely upon the amount of light prevailing during growth. The number of living leaves for the plants in the hot climate reached a definite plateau by August 20, 1952 (Figure 4). In all climates there was a decrease in the number of living leaves starting August 20 (Figures 2, 3, and 4), but this decrease was relatively minor for the plants in the cold and hot climates (Figures 2 and 4). Three weeks later, the number of living leaves for the plants in the mild climate increased again (Figure 3), whereas those in the hot climate remained constant (Figure 4). As would be expected there was an increase in the number of old leaves removed from the plants during this period, but three weeks later the rate returned to normal for all climates (Figures 2, 3, 4). Since the same individual counted and removed the leaves during the entire experiment, this would suggest that an external factor, such as a decrease in light intensity (temperature, nutrition and watering were constant) must account for the increased death rate of the leaves. Unfortunately light measurements were not made at the time and this possibility cannot be confirmed or disproved. The plants were entirely free of diseases and insects, and therefore these factors cannot account for the increased death rate of the leaves in all three climates. Leaf formation, it is interesting to note, was not affected (Figure 1).

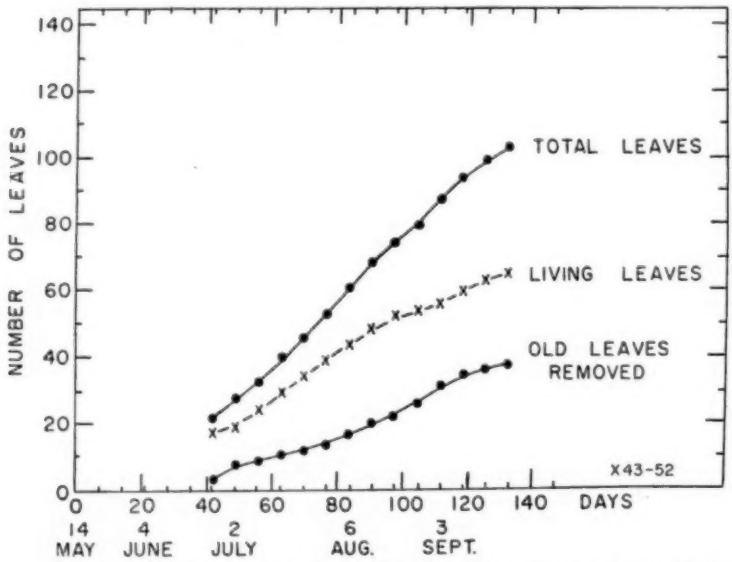


Figure 2.—Relation of time to the total number of leaves formed, the number of leaves living, and the number of old leaves removed from sugar beet plants in a "cold climate" (63° F., 8 a.m. to 4 p.m. and 54° F., 4 p.m. to 8 a.m.). The leaf counts are for two plants per pot, using 12 pots per climate.

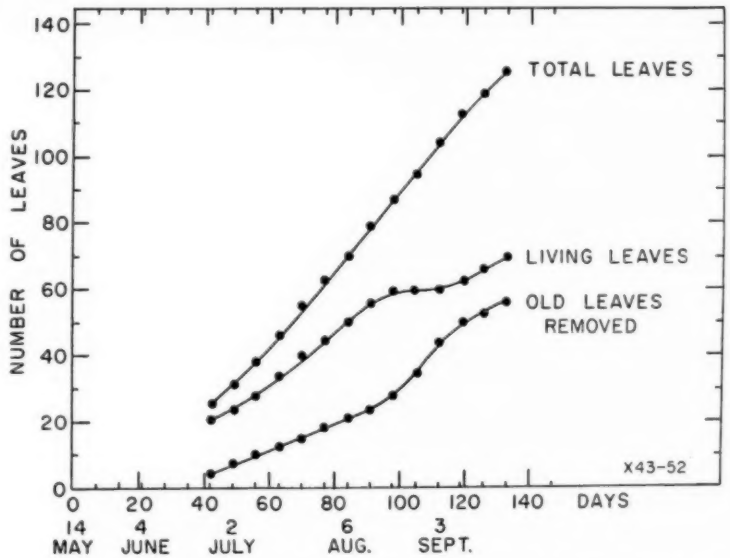


Figure 3.—Relation of time to the total number of leaves formed, the number of leaves living, and the number of old leaves removed from sugar beet plants in a "mild climate" (73° F., 8 a.m. to 4 p.m. and 63° F., 4 p.m. to 8 a.m.). The leaf counts are for two plants per pot, using 12 pots per climate.

Life expectancy of sugar beet leaves: The life expectancy of a sugar beet leaf can be estimated graphically by drawing a line parallel to the abscissa from the old leaf curve to that of the curve for the total leaves formed (Figures 2, 3, and 4). By dropping a line perpendicular to the abscissa from the points of intersection of the two curves, the distance as measured between the two perpendicular lines is the average age of the leaf at the time of death (removal from the plant). When the plants were 126 days old (next to the last point on the curve for the "old leaves removed"), the average age of the leaves before death was 67 days for the plants in the "cold climate," 58 days for the plants in the "mild climate," and only 44 days for the plants in the "hot climate." Thus, while plants grow more slowly in the cold climate, the leaves live much longer and remain functional for a longer time than the leaves in the "mild" or "hot" climates. The shorter life expectancy, and therefore the much smaller tops of the plants in the "hot climate," may be another reason for the smaller beet root growth and the lower sugar concentrations of the beet plants in the "hot climate" in comparison to the plants in the "mild climate." Low temperatures, in contrast to moderate temperatures, retard growth (Table 2) but in terms of sugar produced (Table 4), the smaller beet root size at low temperatures is only partly compensated for by the higher sucrose concentrations of the "cold climate" beet roots (Table 3).

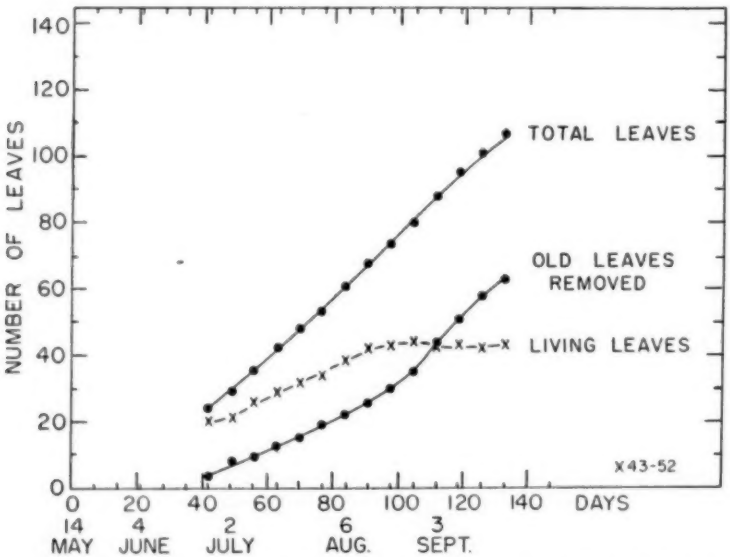


Figure 4.—Relation of time to the total number of leaves formed, the number of leaves living, and the number of old leaves removed from sugar beet plants in a "hot climate" (86° F., 8 a.m. to 4 p.m. and 72° F., 4 p.m. to 8 a.m.). The leaf counts are for two plants per pot, using 12 pots per climate.

Summary

Sugar beet plants of the variety U. S. 22/3 were grown by the open pot culture technique, using vermiculite as the solid medium, and a complete nutrient solution added daily in excess as the liquid medium. Nine sets of plants (three per climate) were germinated and grown in vermiculite for the first 56 days in a controlled temperature greenhouse simulating either a cold, mild or hot climate. After 56 days, one set of the three was retained in the original climate and the two sets remaining were exchanged for plants in the other two climates. These plants were then grown for another period of 75 days.

The climatic sequences produced the following effects:

(a) Beet root growth was influenced by the initial climate and still more by the final climate. A mild (temperate) climate was conducive to root growth whether this occurred early or late in the growing season. A hot or a cold climate, occurring at either time, reduced growth.

(b) Sucrose concentrations were influenced primarily by the final climate. No initial climate effects were detectable. Climatic measurements (temperature, light) made prior to harvest should anticipate the sucrose concentration of the crop. A cold climate was found conducive to high sucrose concentrations and a hot climate to low sucrose concentrations.

(c) The sucrose stored by the beet root reflected the combined effects of climate on root size and sucrose concentration. Temperate weather was conducive to sucrose storage, whether this occurred early or late in the growing season. Cold weather was conducive to sugar storage, providing it was associated with temperate weather. Early hot weather was not nearly so detrimental as hot weather late in the season.

(d) The tops of the sugar beet plants remained green and tended to increase in size during cold weather. During hot weather the tops decreased in size, whether the hot weather occurred early or late in the growing season. Blade weights, however, were affected primarily by the final climate. Hot weather during the final growth period was found to be detrimental to blade growth. Old leaf formation (dead leaves) was in accord with these findings.

(e) The ratio of blades to total top weight, on the fresh weight basis, was the highest for plants transferred to the cold climate from either the mild or hot climates. No other combinations of climate differed significantly.

(f) The leaves of sugar beet plants in a cold climate were characterized by a dark green color and by short, stocky petioles and by broad, thick blades. The blades of plants in a hot climate were a pale green color and were long and narrow in shape, with the outer edges of the blades curled upward. The petioles of these leaves were a pale yellow to almost white in color and were long, narrow, and brittle in structure. A change in climate was followed by a change in kind of leaf formed. The new leaves formed assumed the appearance characteristic of the new climate regardless of the original climate.

(g) The rate of new leaf formation was not affected by climate nearly as much as leaf appearance or the number of living leaves present at any one time. In a cold climate, the living leaves gradually increased in number so that they exceeded greatly the number of living leaves of plants in a hot climate. The life span of a sugar beet leaf in a cold, mild or hot climate was approximately 67, 58 or 44 days, respectively.

The present investigation was undertaken at the Earhart Plant Research Laboratory in Pasadena, California, with the California Institute of Technology, the University of California, and the Beet Sugar Development Foundation cooperating. The active support of these organizations and the aid of many individuals, particularly Dr. F. W. Went and Mr. Kenneth Ohki, is gratefully acknowledged.

References

(1) BROWNE, C. A., and ZERBAN, F. W. 1941. Physical and chemical methods of sugar analysis. John Wiley and Sons, Inc., New York.

(2) HOAGLAND, D. R. and ARNON, D. I. 1950. The water-culture method for growing plants without soil. Calif. Agr. Exp. Sta. Cir. 347:1-32.

(3) ULRICH, A. 1952. The influence of temperature and light factors on the growth and development of sugar beets in controlled climatic environments. Agr. Journ. 44:66-73.

(4) ULRICH, A. 1955. Influence of night temperature and nitrogen nutrition on the growth, sucrose accumulation and leaf minerals of sugar beet plants. Plant Physiol. 30:250-257.

(5) WENT, F. W. 1949. The Earhart Plant Research Laboratory. Chronica Botanica. Boston, Mass.

Nitrogen Constituents Associated with Reduction of Sucrose Percentage and Purity of Sugar Beets[1]

J. L. Haddock, D. C. Linton, and R. L. Hurst[2]

The role of nitrogen fertilizer is associated intimately with the efficient production of sugar beets. Early in 1955 the senior author circulated a questionnaire to determine the fertilizer practices being followed in the principal sugar beet growing areas of western United States. Prominent among the fertilizer problems in need of solution in all areas was the proper use of nitrogen.

This general interest in nitrogen stems from the realization that nitrogen deficiencies result in relatively low yields, while excesses of nitrogen frequently give high tonnages of beets relatively low in sugar content and purity. Factory operators have become increasingly conscious of the nitrogen problem as they struggle with ways of increasing sugar recoveries from sugar beet extractions.

Studies by Afanasiev et al. (1)[3], Gardner and Robertson (4), Haddock (5), Hirst and Greaves (7), Larson (8), Walker and Hac (12), Walker and Hac (12), and Ulrich 10), among others, have shown a close inverse relation between nitrogen fertilization and sugar beet quality. The relation between these two factors is partially explained by Walker and Hac (12) on the basis of growth and storage potential and by Ulrich (10) on the basis of environmental factors such as intensity of sunlight, night and day temperatures, and nitrogen supply.

It should not be assumed that nitrogen fertilization is the only factor which may modify the sucrose and purity of sugar beet extracts. It is suggested by Brown and Wood (2) and Doxtator and Bauserman (3) that sodium and potassium may be melassigenic substances.

Since it has been shown that nitrogen fertilization and nitrogen content of plant tissue are related to depression of sugar and purity of the extract juice of beet roots, it seems desirable to determine which of the various nitrogen constituents in beet roots appears to be related directly to these quality factors.

Procedure

The experimental material used in this study was obtained from an extensive field experiment on Millville loam at Logan, Utah. Two methods of irrigation, sprinkler and furrow, with four soil moisture conditions superimposed on each method, were studied (available soil moisture remaining in root zone allowed to approximate 90 (W_4), 70 (W_3), 40 (W_2), and 20 (W_1) percent between irrigations). Soil moisture plots were further subdivided for nitrogen and phosphorus fertilizer treatments. Nitrogen was applied at the rate of 80 pounds N per acre as ammonium sulfate.

---

[1] Contribution from the Soil and Water Conservation Research Branch, ARS, USDA, Western Regional Research Project W-29, and the Utah-Idaho and Amalgamated Sugar Companies, and the Utah Agricultural Experiment Station, cooperating.

[2] Soil Scientist and Chemist, Western Soil and Water Management Section, Soil and Water Conservation Research Branch, ARS, USDA, and Director of Statistical Laboratory, Utah Agricultural Experiment Station, respectively.

[3] Numbers in parentheses refer to literature cited.

The pulp samples were dried for 48 hours in a forced draft oven at 70° centigrade. All analyses, with the exception of sucrose, purity, and glutamine, (made on fresh pulp) were made on dried pulp. Glutamic acid was determined by the biological assay procedure on frozen fresh pulp and calculated to dry basis. Glutamine nitrogen was calculated from glutamic acid data. The analytical chemical procedures were carried out as follows:

1. Total organic and ammoniacal nitrogen were determined by a slight modification of the method of Hillebrand et al. (6). Four grams of digestion mixture (500 parts Na_2SO_4 50 parts $CuSO_4$ and 5 parts Se were added to solution containing 0.5 gram of dry beet pulp. Fifteen ml. H_2SO_4 were added and the mixture boiled until clear plus 5 minutes. Solution was made alkaline and distilled.

For all other determinations 4 grams of the dried pulp was extracted with 140 ml. 2 percent acetic acid solution and 50 ml. aliquots were taken for the different analyses as follows:

2. Soluble organic and ammonical nitrogen were made as in 1 above.

3. Ammonia nitrogen was determined by direct Nesslerization of an aliquot of extract (14).

4. Soluble organic nitrogen = procedure 2 minus 3 above.

5. Ammonia plus amide nitrogen was determined by distilling an aliquot from a 20 percent NaOH solution and titrating (13).

6. Nitrate nitrogen was determined by adding Devardas Alloy to the residue from procedure 5 and distilling (6).

7. Amide nitrogen = procedure 5 minus 3.

8. Amine nitrogen (this may also include nitrogen from pyridine type compounds) = procedure 2 minus 5.

9. Soluble nitrogen = procedure 2 plus 6.

Table 1.—Yield and Quality of Sugar Beet Roots as Affected by Various Treatments (1950).

Treatments		Net Water Applied	Yield T/A	Dry Matter Percent	Sucrose Percent	Purity Percent
Soil Moisture Condition						
Dry	W_1	16.3	14.74	22.90	16.05	88.38
Mod-dry	W_2	18.3	17.75	23.62	16.73	89.80
Mod-moist	W_3	26.3	19.20	23.91	17.01	90.28
Moist	W_4	50.9	18.38	23.99	17.06	90.80
s.e. of mean N = 32			.99	.21	.24	.48
Method						
Sprinkle		24.5	17.93	23.34	16.43	89.03
Furrow		31.4	17.10	23.87	16.99	90.60
L.S.D. @ .05			N.S.	0.36	0.36	0.71
Fertilizer						
Nitrogen		27.9	18.35	23.46	16.45	88.69
No Nitrogen		27.9	16.68	23.75	16.98	90.95
L.S.D. @ .05			0.69	0.22	0.04	0.35

Sugar and purity determinations were made by cold water extraction procedure.

Experimental Results

Data on yield and quality of sugar beets are presented in Table 1. It will be observed that yield, dry matter, sucrose, and purity are all affected to a significant extent by the variation in soil moisture condition used in the experiment. It will be noted that while yield is not affected significantly by method of irrigation the three quality factors are affected significantly. In agreement with work cited previously nitrogen fertilizers do affect the quality of the beets as well as the yield.

It will be well to keep in mind that quality (sucrose and purity) tends to increase as soil moisture is increased; that quality is higher under furrow than sprinkle irrigation; and that quality is lower under nitrogen fertilization than without it.

Table 2.—Nitrogen Constituents of Sugar Beet Roots as Affected by Various Treatments (1950).

Parts Per Million, Dry Basis

Treatments	Nitrogen Constituents in 2 Percent Acetic Acid Extract						Nitrogen in Whole Pulp	
	Nitrate	Ammonia	Amine	Amide	Organic	Soluble	Glutamine[1]	Total
Soil Moisture Condition:								
Dry W_1	762	145	3089	796	3885	4789	1738	7968
Mod-dry W_2	531	119	2676	685	3361	4006	1448	6932
Mod-moist W_3	571	106	2525	645	3103	3787	1246	7169
Moist W_4	521	82	2262	532	2776	3378	1018	6455
s.e. of mean n = 32	91	16	193	N.S.	235	86	126	344
Method:								
Sprinkle	626	132	2868	724	3593	4343	1602	7505
Furrow	566	94	2409	605	2969	3637	1114	6757
L.S.D. @ .05	N.S.	24	284	N.S.	345	126	186	505
Fertilizer:								
Nitrogen	657	157	3194	799	3988	4804	1790	8067
No Nitrogen	535	69	2082	530	2575	3176	926	6195
L.S.D. @ .05	85	12	215	84	202	73	93	293

[1] Data used here by courtesy of International Minerals and Chemical Corporation.

One should notice the general trends of various nitrogen constituents as affected by the irrigation and fertilizer treatments shown in Table 2, keeping in mind the trends in quality observed in Table 1. Here it is seen that all the nitrogen constituents with one minor exception are inversely related to the quality factors shown in Table 1. Also with the exception of amide as affected by irrigation regimes and nitrate nitrogen concentration as modified by method of irrigation, it will be noted that the treatments affect nitrogen content of beet roots significantly.

The data in Tables 1 and 2 mean that any essential change in irrigation or fertilizer practice may modify the nitrogen nutritional status of sugar beets with respect to all the nitrogen constituents, and further, that this change in nutritional status may be associated with changes in sugar beet quality.

The authors recognize the possibility of sugar beets absorbing part of their nitrogen as ammonia, nitrates, and even organic nitrogen from the soil, but assume with Nightingale (9) that under usual field conditions the principal source of nitrogen is nitrates. Apparently, when nitrates are plentiful in the substrate and concentrations become high in beet roots, the reduced form of inorganic nitrogen or ammonia increases correspondingly. When the ammonia concentration increases in the root the ammonia detoxication agents such as glutamine similarly increase. This statement is substantiated by the data in Table 2 and is in agreement with the findings of Vickery et al. (11).

It might be assumed from what has been said that the ratio of one nitrogen constituent in the sugar beet root to any other should be relatively constant. If this were true and if sugar beet quality were closely related to soluble nitrogen constituents, it may be possible to establish a relation of association between beet quality and any convenient nitrogen constituent. It does not appear possible from a cursory examination of the data in Tables 1 and 2 to determine which of the nitrogen constituents is most closely associated with or responsible for changes in sugar beet quality. This may be done by comparing the ratios of one constituent to another and by comparing this ratio with changes in quality observed in Table 1.

Although insoluble protein nitrogen is not listed in Table 2, it can be obtained by deducting soluble nitrogen in the acid extract from total

Table 3.—Ratios Among Some Nitrogen Constituents in Sugar Beet Roots as Affected by Various Treatments (1950).

Treatment		Protein Soluble	Protein Sol. Org.	Protein Inorg.	Protein Gluta-mine	Amine Gluta-mine	Nitrate Gluta-mine	Amide Gluta-mine	Ammonia Gluta-mine
Soil Moisture Condition									
Dry	W_1	.75	1.00	4.22	2.15	1 89	.472	.504	.082
Mod-dry	W_2	.92	1.09	7.04	3.08	2.24	.435	.541	.080
Mod-moist	W_3	1.01	1.22	7.20	3.57	2.46	.578	.611	.082
Moist	W_4	1.09	1.43	6.44	5.41	3.17	.819	.611	.101
s.e. of mean n = 32		.11	.18	.83	.73	.35	.11	N.S.	N.S.
Method									
Sprinkle		.87	1.09	5.83	2.68	2.09	.442	.473	.085
Furrow		1.01	1.28	6.62	4.41	2.78	.709	.661	.088
L.S.D. @ .05		N.S.	N.S.	N.S.	1.07	.52	.165	N.S.	N.S.
Fertilizer:									
Nitrogen		.78	.94	5.67	2.13	1.89	.377	.455	.084
No nitrogen		1.10	.143	6.78	4.96	2.99	.775	.679	.088
L.S.D. @ .05		.17	.27	N.S.	0 82	.35	.143	.104	N.S.

nitrogen. This is a relatively constant value of approximately 3140 p.p.m. under all conditions of the experiment. This form of nitrogen is relatively stable and it is probably the most satisfactory basis to use in studying the ratio changes in other constituents. Furthermore, since it is insoluble it probably has less influence on the quality factors of sucrose and purity than soluble constituents. Some of the ratios of protein to soluble nitrogen fractions are shown in Table 3. Although these ratios vary within relatively narrow limits they are regular and consistent with changes in sugar beet quality as affected by irrigation and fertilizer treatments (see Table 1). This increase in rotio with increase in quality indicates that the soluble constituents are varying inversely with changes in quality. Columns 5, 6, 7, and 8 in Table 3 indicate similarly that the concentration of glutamine in the beet root decreases more rapidly than does amine or amide nitrogen and that glutamine also decreases more rapidly than nitrate or ammonia nitrogen as quality increases. The ratio of ammonia to glutamine does not follow a consistent change as beet quality changes. This indicates a close relation between ammonia and glutamine concentrations in sugar beet roots.

If one were to decide from the data in Table 3 which of the nitrogen constituents in the sugar beet root were most closely associated with variations in quality, it appears that glutamine and ammonia nitrogen would be selected. In order to determine more specifically which of the nitrogen constituents were most closely associated with the quality of roots correlation coefficients were calculated. Simple correlations among the various nitrogen concentrations are shown in Table 4.

Table 4.—Simple Correlations Among Nitrogen Constituents of Sugar Beet Roots.

	Nitrate	Amine	Amide	Ammonia	Organic	Total	Total Soluble
Glutamine	.0956	.6337	.4173	.7284	.7758	.6271	.7547
Nitrate	.....	.1234	.0422	.0642	.1291	.1353	.3001
Amine	.....	.....	.1430	.4667	.9085	.4424	.8690
Amide	.....	.....	.....	.2678	.3589	.2184	.3459
Ammonia	.....	.....	.....	.....	.5686	.5261	.5693
Organic	.....	.....	.....	.....	.....	.5137	.9518
Total	.....	.....	.....	.....	.....	.....	.5415
Total Soluble	.....	.....	.....	.....	.....	.....	.....
n = 128		Sig. @	.05	0.174			
			.01	0.228			

The high correlations between soluble and organic as well as organic and amine is explainable on the ground that organic and amine constitute approximately eighty percent of the soluble and organic fractions respectively. Some of the other high correlations may be explained in a similar manner. The high correlation between glutamine and ammonia are associated on a different basis.

The data in Table 5 consist of simple regression coefficients indicating the association of change in nitrogen fractions with changes in yield and quality factors. There is a tendency for yields to increase as nitrogen con-

stituents increase. The data show a highly significant inverse relation among quality factors and all nitrogen constituents.

Since it has been shown that all nitrogen constituents tend to vary inversely with quality of sugar beet roots when studied together, it was thought advisable to test the theoretical relation of each nitrogen constituent to quality, assuming that all other nitrogen constitutents were not permitted to vary. These partial regression coefficients are shown in Table 6. Under these theoretical conditions, it will be observed that glutamine is significantly related to dry matter and sucrose and approaching significance to purity. Ammonia nitrogen appears to be the only other fraction significantly related to quality.

The data in Table 7 were prepared in order to determine the percent of the total variation in quality in beet roots associated with nitrogen constituents. It will be seen that the largest variation in root quality associated with nitrogen composition is attributable to the presence of

Table 5.—Simple Regression Coefficients Relating Yield and Quality Factors to Nitrogen Fractions in Sugar Beet Roots.

Nitrogen Constituents p.p.m.	Yield T/A	Dry Matter Percent	Sucrose Percent	Purity Percent
Glutamine	+.0051	—.0070[2]	—.0091[2]	—.0289[2]
Nitrate	—.0023	—.0103[2]	—.0103[2]	—.0237[2]
Amine	+.0043	—.0031[2]	—.0047[2]	—.0162[2]
Amide	+.0029	—.0104[2]	—.0126[2]	—.0391[2]
Ammonia	+.0029	—.0611[2]	—.0792[2]	—.2241[2]
Organic	+.0034	—.0032[2]	—.0045[2]	—.0150[2]
Total	+.0036	—.0020[2]	—.0028[2]	—.0098[2]
Total Sol.	+.0025	—.0030[2]	—.0041[2]	—.0129[2]

[1] Significant beyond .05 level n = 128
[2] Significant beyond .01 level

Table 6.—Partial Regression Coefficients relating Yield, Quality Factors, and Nitrogen Constituents in Sugar Beet Roots.

P.P.M.	Yield T/A	Dry Matter Percent	Sucrose Percent	Purity Percent
Glutamine	+.0047	—.0078[1]	—.0067[2]	—.0024
Nitrate	—.0883	—.0119	—.0066	+.0024
Amine	—.0082	+.0023	+.0014	—.0040
Amide	—.0147	—.0009	—.0008	—.0069
Ammonia	—.2485[1]	—.0326	—.0348[1]	+.0163
Organic	—.0642[2]	—.0039	—.0017	+.0085
Total	+.0065	+.0012	+.0009	—.0006
Total Sol.	+.0775	+.0038	+.0008	—.0068
R^2	.094[2]	.323[2]	.559[2]	.783[2]

n = 128

[1] Sig *6t* .05
[2] Sig. *6t* .01

glutamine. Associated with this constitutent are 23 percent of the variation in dry matter, 50 percent of the sucrose, and 77 percent of the purity. While ammonia accounts for a high percent of variation, it is less closely associated with quality than is glutamine nitrogen. A comparison of variations in quality associated with all nitrogen constituents (R^2), in Table 7, shows that glutamine is associated with or can account for 73 percent of the total variation in dry matter, 89 percent in sucrose, and 98 percent in purity. It may be interesting to note that nitrate nitrogen is associated with a relatively low percentage of the total variation.

Table 7.—Simple Correlation Coefficients Relating Yield and Quality Factors to Nitrogen Fractions of Sugar Beet Roots (r^2).

P.P.M.	Yield T/A	Dry Matter Percent	Sucrose Percent	Purity Percent
Glutamine	.009	(—).235	(—).497	(—).769
Nitrate	(—).000	(—).117	(—).147	(—).119
Amine	.015	(—).102	(—).297	(—).541
Amide	.001	(—).134	(—).247	(—).361
Ammonia	.000	(—).210	(—).446	(—).545
Organic	.013	(—).153	(—).389	(—).651
Total	.027	(—).116	(—).285	(—).516
Total Sol.	.010	(—).189	(—).429	(—).654
R^2	.094[2]	.323[2]	.559[2]	.783[2]

n = 128 Sig. of .05 0.0303
.01 0.0519

Summary and Conclusions

Numerous observations have shown that the problem of nitrogen fertilization is a prominent one in the sugar beet growing areas of the United States. Yields and quality of sugar beets are so markedly affected by the quantity of available soil and fertilizer nitrogen that neither a deficiency nor a large excess should be tolerated.

Although factors of climate and soil, other than nitrogen, may affect the quality of sugar beet roots, it cannot be denied that nitrogen fertilization and nitrogen plant composition are closely associated with sucrose storage in beet roots and sugar recoveries from extract juice.

The authors do not contend that nitrogen constituents per se prevent sugar crystallization, but there is strong evidence that soluble nitrogen constituents of the sugar beet root are highly associated with, if not responsible for, variations in purity and sucrose percentage as well as dry matter percentage.

The particular nitrogen components which appear to be most highly related to changes in sugar beet quality are the glutamine and ammonia fractions. It is the opinion of the authors that glutamine nitrogen is of greatest significance in quality variation, not only because of its high association with quality factors, but also because the concentration of nitrogen in this form is ten times that of ammonia nitrogen.

References

(1) Afanasiev, M. M., Frahm, E. E., Morris, H. E., and Johnston, W. B. 1953. Physiology of growth, sugar accumulation and mineral intake of sugar beets in Montana. Montana Agr. Exp. Sta. Tech. Bul. 491.

(2) Brown, R. J. and Wood, R. R. 1952. Improvement of processing quality of sugar beets by breeding methods. Proc. Amer. Soc. Sugar Beet Tech. pp. 314-318.

(3) Doxtator, C. W. and Bauserman, H. M. 1952. Parent-progeny tests for sodium and potassium content. Proc. Amer. Soc. Sugar Beet Tech. pp. 319-321.

(4) Gardner, Robert and Robertson, D. W. 1942. The nitrogen requirement of sugar beets. Colo. Agr. Exp. Sta. Tech. Bul. 28.

(5) Haddock, Jay L. 1954. The interrelationships of irrigation method, soil moisture condition and soil fertility on the yield, quality and nitrogen nutrition of sugar beets. Symposium in Plant Analysis and Fertilizer Problems. Eighth International Botanical Congress pp. 121-135.

(6) Hillebrand, W. F., Bright, H. A., Hoffman, J. I., and Lundell, G. E. F. 1944. Kjeldahl method pp. 633-641. Applied Inorganic Analysis. John Wiley and Sons, New York. pp. 783-788.

(7) Hirst, C. T. and Greaves, J. E. 1944. Noxious nitrogen in leaves, crowns and beets of sugar beet plants grown with various fertilizers. Soil Sci. 57:417-424.

(8) Larson, W. E. 1952. Fertilizer studies with sugar beets in south central Montana. Proc. Amer. Soc. Sugar Beet Tech. pp. 237-243.

(9) Nightingale, Gordon T. 1937. The nitrogen nutrition of green plants. The Botanical Review 3:85-174.

(10) Ulrich, Albert. 1942. The relationship of nitrogen to the formation of sugar in sugar beets. Proc. Amer. Soc. Sugar Beet Tech. pp. 66-80.

(11) Vickery, H. B., Pucher, G. W., and Clark, H. E. 1936. Glutamine metabolism of the beet. Plant Physiol. 11:413-420.

(12) Walker, Albert C. and Hac, Lucile R. 1952. Effect of irrigation practices upon the nitrogen metabolism of sugar beets. Proc. Amer. Soc. Sugar Beet Tech. pp. 58-66.

(13) Wertheim, E. Textbook of Organic Chemistry, Second edition pp. 228. P. Blakiston's Son & Co. Inc., Phila.

(14) Woodman, A. G. and Norton, J. F. 1914. Air, Water and Food, Fourth Edition. pp. 77. John Wiley and Sons Inc.

Silver Engineering's New Grab-Roll Beet Screen

CLARENCE R. STEELE[1]

Changing harvest conditions have increased the demands on beet screens until it has become imperative to have a screen that is more effective with the large quantities of dirt, trash, and clods that we now receive.

Last spring several of you repeated your demands for a better method of cleaning beets. We, at Silver Engineering, went into a huddle and reviewed all that we knew of how to, and how not to screen beets and started experimenting again.

We made some crude models. We dug clods. We mixed mud. Great Western donated all the beets they could spare from their Longmont cellars. When we wore them out, we brought carrots and parsnips. We finally came up with a screen element which, under the conditions we could create in our shop, would do the job and which we were sure would do the job in the field.

Harvest was then in full swing in the Imperial Valley and Holly offered the use of their Heber dump. This dump had on it a double reverse Rienks screen. This Rienks screen was 60 inches wide and had 9 rolls, including 3 reversing rolls. We designed and built one of our new screens for it. We had to have one which we could put in over night so we had to compromise somewhat. The screen was both narrower and shorter than we would have liked, but it was a good trial unit.

This screen was installed June 30 and put to work the next day. At this dump they were receiving beets containing large quantities of moist, tough clods. Tables 1 and 2 show comparative data between the old screen and the new one under these cloddy conditions. You will note the tremendous improvement in the screening efficiency.

Union Sugar had also worked closely with us at Heber and they decided to install one of these new screens on their Sargent dump for the fall campaign. Since the two dumps were nearly identical, the screen for the Sargent dump was almost the same as the Heber screen. We made only a few small changes to incorporate some of the things we learned at Heber.

Table 1.—Heber, California. Comparison of Last Day of Operation of Old Screen with First Day of New Screen. Approximately 350,000 Pounds of Beets Each Day from the Same Field.

Gross Weight Beets	Old Screen 100	New Screen 100
Net Weight Clean Beets	76.55	**81.04**
Total Screenings Plus Tare	23.45	18.96
Tare	18.92	8.73
Attached Crowns and Tops	4.59	4.86
Total Possible Screenings	18.86	14.10
Actual Screenings	4.53	10.23
Screen Effectiveness	24.02%	72.55%

[1] Vice President, Silver Engineering Works, Inc., Denver, Colorado.

Table 2.—Heber, California. Comparison of Screens with Approximately 2,000,000 Pounds of Beets Over New Screen and With Approximately 1,500,000 Pounds of Beets Over Old Screen.

Gross Weight Beets	Old Screen 100	New Screen 100
Net Weight Clean Beets	80.97	80.70
Total Screenings Plus Tare	19.03	19.30
Tare	14.08	9.96
Attached Crowns and Tops	4.86	4.84
Total Possible Screenings	14.17	14.46
Actual Screenings	4.95	9.34
Screen Effectiveness	34.93%	64.59%

Table 3.—Sargent, California. Comparison of Screens With Approximately 40,000,000 Pounds Over New Screen and Approximately 35,000,000 Pounds Over Old Screen.

Gross Weight Beets	Old Screen 100	New Screen 100
Net Weight Clean Beets	89.43	88.88
Total Screenings Plus Tare	10.57	11.12
Tare	7.67	5.72
Attached Crowns and Tops	4.47	4.44
Total Possible Screenings	6.10	6.68
Actual Screenings	2.90	5.40
Screen Effectiveness	47.54%	80.84%

The screen was installed about the middle of their harvest. Table 3 shows comparative data for the old and new screens at Sargent. These data cover all of the beets received during a three-month harvest season. Again, you will note the improvement in screening efficiency.

This new screen is composed of several rolls of Rienks kickers, all turning forward, followed by a series of grab-roll units. Figure 1 shows a standard screen arrangement. The Rienks rolls screen out the loose, easily-removed dirt and leaves the grab-rolls free to concentrate on the difficult material. They also spread the beets sideways. The first three rolls are rubber to assist the spreading. The last roll is steel to prevent the loss of beets between it and the first smooth roll.

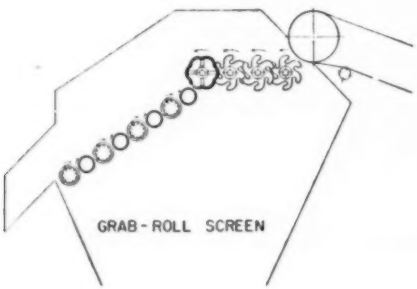


Figure 1.—Diagram of a standard screen arrangement.

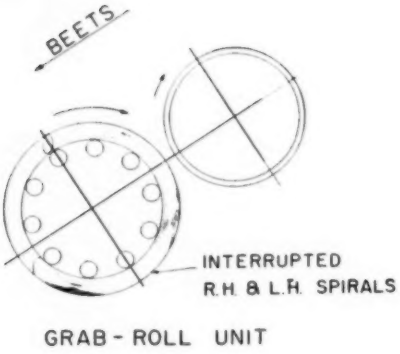


Figure 2. — Diagram of a grab-roll unit.

The grab-rolls are mounted on a slope and all turn upward against the flow of beets. A grab-roll unit is shown in Figure 2.

Practically all of the removal of foreign matter by the grab-rolls takes place through the opening above the cage roll. The corrugated-like surface of this roll plus the fact that it turns approximately four times the speed of the smooth roll tends to drag material into this opening. Material on which this roll can get a bite is immediately dragged through. The upward motion of the smooth roll controls the degree of bite to prevent damage to the beets. There is a selective action here between beets and foreign matter. The rolls will bite a clod, mud ball, or trash ball but not a beet.

A few beets will get tail-down into this opening. The controlling action of the smooth roll keeps them from being pinched, but even so they must then be removed or the screen will soon be plugged. This is accomplished by the spirals on the cage roll.

These spirals are in short sections and on each cage roll are alternately right hand and left hand. They are offset so that the adjacent runout points of the right and left hand sections are 180 degrees apart.

The beet that gets between the rolls is conveyed rapidly sideways by a spiral. When this spiral runs out, the beet overtravels and the opposing spiral strikes it to throw it back. With a beet the center of mass is well above the rolls. This means that the spiral strikes it below its center which flips the beet upside down and out of the rolls.

Again, there are selective actions here. Any piece of foreign matter which is too big to be pulled through at the first bite will also be caught by the spiral, but this cannot be conveyed sideways as easily as a beet; thus, there is a chance that the spiral will break it or drag it through. If it is not broken, it will be conveyed sideways by the spiral the same as a beet; but unlike the beet its center of gravity is low so that when the opposing spiral strikes it, the blow will drive it straight back along the roll and not flip it out.

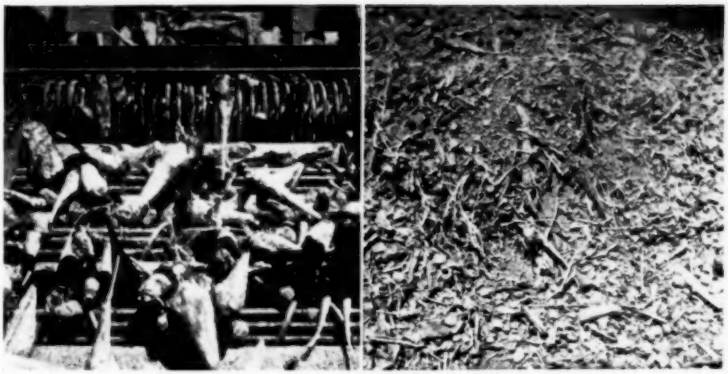


Figure 3.—Beets and clods on a grab-roll screen at Herber, California, are shown at the left and screenings from a grab-roll screen are shown on the right.

These actions result in dirt, clods, trash, etc., being held next to the rolls until they are broken up and taken through while beets dance on over the screen.

This year we designed a 42-inch piler. The design was new from the ground up. We reviewed every part of it, every detail, every function. We incorporated every improvement that would make it more convenient, faster and more effective. These included open construction for greater accessibility, hydraulic dumping strut, hydraulic platform and piler lifting, hydraulic steering, more complete and direct dirt recovery, automatic tare-taker, power boom raising, power boom swinging and automatic oscillation and, of course, a grab-roll screen.

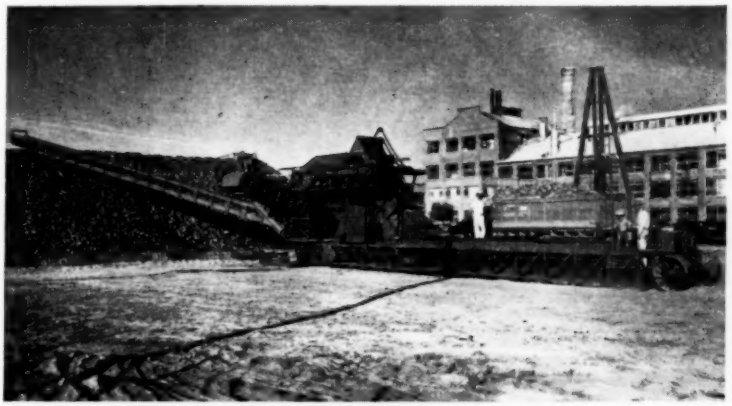


Figure 4.—Forty-two inch piler at Tracy, California.

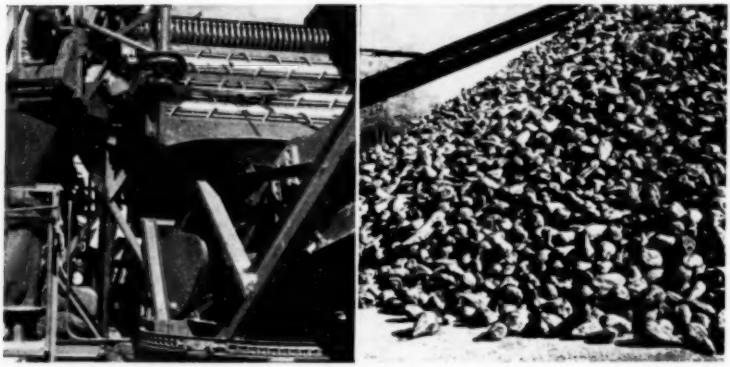


Figure 5.—A grab-roll screen on a 42-inch piler shown on the left and a beet pile at Tracy, California, from a 42-inch piler shown on the right.

This screen was 90 inches wide and consisted of 7 Rienks rolls and 5 sets of grab-rolls.

The first piler of this design is in the factory yard of the Holly Sugar Corporation at Tracy, California. Many of you have seen it. During the early part of its operation, before it started to rain, they were able to restrict their piling to just the cleanest loads. Table 4 shows the screen performance on approximately 1,750,000 pounds of the beets handled during this dry weather. You will note that the screen removed 87.83 percent of the removable material. There is no question in my mind but that the pile put up with this piler was the cleanest pile ever put up with mechanically harvested beets.

After it started to rain, they increased the speed of the harvest to finish as quickly as possible; thus, they took beets over the piler without selection.

This was our first field experience with muddy beets. We had tried mud in the shop and found no difficulty. There had been enough moisture at Heber to cause some dirt to stick to the rolls. In both cases we found that the differential speed between the two rolls had a cleaning action that limited buildup. Nevertheless, we were anxious to try really muddy beets and at last our hopes were realized.

There had been some loose mud balls forming inside the cage rolls. When we got into the mud, these rolls filled up solid and then quit building. Very little mud stuck to the smooth rolls, but, where it did stick, the kneading action of the spiral on the cage roll caused it to peel off. No cleaning was necessary and the screening action was as good as ever. There was some excessive buildup in the Rienks kickers and they required some cleaning.

During the last two weeks in November, Tracy had .84 inches of rain. Table 4 shows the screen performance on approximately 2,000,000 pounds

of beets handled during that time. You will note that even under these conditions the screen still removed 75.25 percent of the removable foreign matter.

The Heber and Sargent screens had 4 Rienks rolls and 3 sets of grab-rolls. The Tracy screen had 7 Rienks rolls and 5 sets of grab-rolls. After observing these screens, taking into account their performance with trash, clods, and mud, and considering construction costs, we believe the standard screen should have 4 Rienks rolls and 4 sets of grab-rolls as shown in Figure 1. A screen for a 42-inch conveyor should be 90 inches wide; for a 36-inch conveyor—72 inches wide; and for a 30-inch conveyor—60 inches wide.

This grab-roll screen has demonstrated that with tough clods it will break them and screen them out. It has demonstrated that it will remove large quantities of trash—even clumps of weed roots. It has also demonstrated an ability to cope with sticky mud.

We, at Silver Engineering, are proud of it and confident that it will go a long way toward meeting your demands.

Table 4.—Tracy, California. 42-Inch Piler Screening Results on Approximately 1,750,000 Pounds of Beets During Dry Weather.

Gross Weight Beets	100
Net Weight Clean Beets	97.33
Total Screenings Plus Tare	7.91
Tare	5.23
Attached Crowns and Tops	4.87
Total Possible Screenings	3.04
Actual Screenings	2.67
Screen Effectiveness	87.83%

Table 5.—Tracy, California. 42-Inch Piler Screening Results on Approximately 2,000,000 Pounds of Beets During 2 Weeks of Rainy Weather.

Gross Weight Beets	100
Net Weight Clean Beets	80.86
Total Screenings Plus Tare	19.14
Tare	10.11
Attached Crowns and Tops	7.14
Total Possible Screenings	12.00
Actual Screenings	9.03
Screen Effectiveness	75.25%

Treatment of Sugar Beet Seed with Systemic Insecticides for Control of the Beet Leafhopper[1]

ORIN A. HILLS, F. H. HARRIES, AND A. C. VALCARCE[2]

Control of the beet leafhopper is essential on susceptible varieties of sugar beets grown for seed in the Southwest. This was originally accomplished with a pyrethrum-in-oil spray (1)[3], but DDT has been the accepted method since it became available in 1945 (2). DDT remains active for some time on the larger plants, killing leafhoppers that may move into the field after application. However, control is more difficult on cotyledon and two-leaf beets because of lack of sufficient foliage to hold the insecticides. Seedlings are also more susceptible to curly top than the larger plants. Recent research has, therefore, been directed toward the development of better methods for controlling the beet leafhopper on these seedling beets.[4]. This work is still in progress and the results presented in this paper should be considered a progress report.

In the last few years several systemic insecticides have passed the experimental stage and are on the market. Probably the one best known is Demeton (in Systox). These insecticides enter the circulatory system of the plant, causing the juices to be toxic to certain insects. The insecticide may be introduced into the soil with the irrigation water or applied as a foliage spray. Recent tests at Phoenix, Arizona, have also shown that some of these insecticides are effective for considerable time if the seed is treated before planting.

Greenhouse Tests

In the Salt River Valley of Arizona, sugar beets grown for seed are planted in late August or September. In anticipation of field-plot experiments, tests were made in the greenhouse during the spring and summer on sugar beets grown in pots or flats.

Insecticides included in these studies were the phosphorous compounds, Demeton, Schradan, and American Cyanamid 3911 and 12008. Emulsions of these materials were tested as soil drenches and preplanting seed treatments. Dusts, prepared by American Cyanamid to contain 50 percent of 3911 or 12008 on powdered carbon, were also tested as seed coatings. The dosages for seed treatment were calculated on the basis of 15 pounds of seed per acre.

Soil Applications.

The first of these tests was with soil drenches. Potted plants were irrigated with water containing various insecticides and later leafhoppers were confined on the leaves in a special type of leaf cage. Each cage contained one leaf on which 10 leafhoppers were confined for 24 hours. Treatments were at the approximate rates of 8 and 16 pounds of the active ingredient per acre. The eight-pound rate was adequate, and no additional benefit was indicated for the higher dosage. As shown in Figure 1, both American

[1] In cooperation with the Arizona Agricultural Experiment Station.
[2] Entomologists, Entomology Research Branch, A.R.S., U. S. Department of Agriculture, Phoenix, Arizona.
[3] Numbers in parentheses refer to literature cited.
[4] The beet plots on which these experiments were conducted were furnished by the Western Seed Production Corporation.

Cyanamid 3911 and 12008 killed practically all the leafhoppers caged on the plants throughout the experiment, whereas the effectiveness of Demeton dropped off rapidly after about 20 days, but was superior to Schradan.

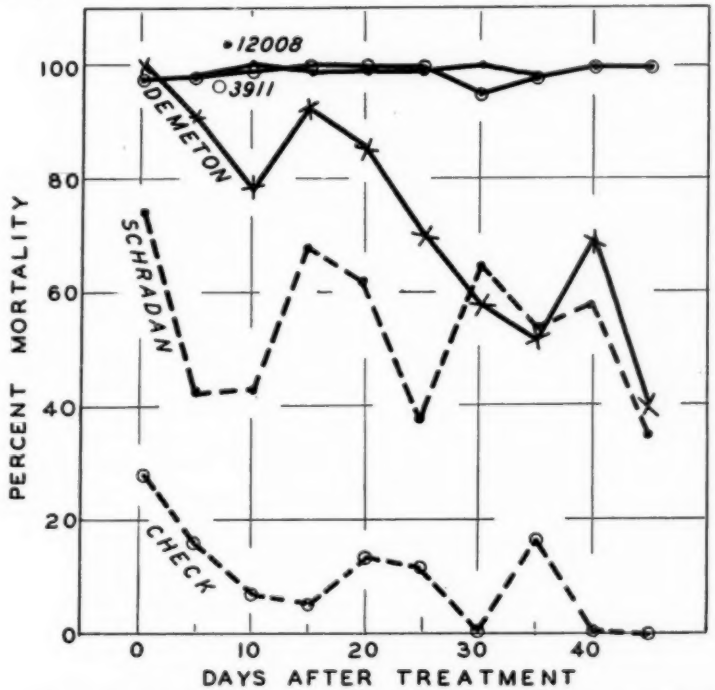


Figure 1.—Mortalities of beet leafhoppers on potted sugar beets subjected to various soil-insecticide treatments. Phoenix, Arizona, 1955.

Seed Treatment.

Tests were also made to determine the effectiveness of seed treatment in the control of leafhoppers on seedling plants. In one test the seed was soaked for 30 minutes in one-percent emulsions of the same materials used in the soil-drench tests. One leafhopper per plant was caged on 25 plants receiving each treatment when the beets were in the four-leaf stage. All the treatments gave good leafhopper control, as shown by the following mortalities:

	Percent
Demeton	100
Schradan	92
American Cyanamid 3911	100
12008	100
Untreated check	20

In another test, the seed was coated with carbon dusts impregnated to contain 50 percent of American Cyanamid 12008 or 3911. By tumbling

in a revolving drum for 15 minutes, approximately 1.1 ounces of the impregnated dust could be made to adhere to one pound of seed. These seeds were planted in six-inch pots and all leafhoppers, caged on the plants 35 days later, died in 24 hours.

Some of the plants from treated seed showed growth abnormalities, therefore, a series of tests was made to determine the plant tolerance of these materials. Seed was treated with various amounts of 12008 or 3911 in 50 percent carbon dusts or various emulsion dilutions. The emulsions were first used as seed soaks, by which the seed was soaked for 30 minutes in emulsions containing 0.5, 1, and 5 percent of the insecticide. There was some indication that the five-percent emulsion reduced the viability of the seed and also caused a high percentage of abnormal plants. Owing to difficulties in treatment and handling of the wet seed, this method was soon abandoned. It was found that a thorough distribution of liquid materials could be obtained in a revolving drum mixer by using only enough water to dampen the seed (1 gallon to 15 pounds of seed) and the quantity of the active ingredient applied to the seed could be more accurately determined. Although the seed was not so wet as when treated by the soak method, there was still the problem of drying it before plantting.

Since the previous tests had shown the two materials to be equally effective against the leafhopper, only 12008 was used in the more concentrated emulsions. Treatments were made at rates of ¼, ½, 1, and 2 pounds to 15 pounds of seed. At ¼ pound, the viability of the seed was satisfactory and plants appeared normal, except for a slight cupping of some of the cotyledons shortly after emergence. At ½ pound, seed viability was considerably reduced and at 1 and 2 pounds, none of the seeds germinated. It was therefore concluded, that by this method, any dosage above ¼ pound to 15 pounds of seed was not safe.

Plant-tolerance tests with carbon dusts impregnated to contain 50 percent of American Cyanamid 12008 or 3911 were made at ⅛, ¼, ½, ¾, and 1 pound of the active ingredient on 15 pounds of seed. Viability of the seed was unaffected up to ¾ pound, but materially reduced at the 1-pound rate. No abnormal plants resulted from the ⅛- or ¼-pound treatment, but approximately one-third of the plants appeared abnormal in the ½-pound treatment and from 63 to 100 percent in the ¾- and 1-pound treatments. The abnormalities in the ½-pound treatment seemed to be confined to the cotyledon and two-leaf stages and as the plants developed these symptoms disappeared.

Field Tests

In the fall of 1955, field tests with treated beet seed were made on a series of small plots and also on two five-acre plots. The rates used on the five-acre plots were within the range considered safe from the greenhouse tests, but in some of the small plots, they were somewhat higher, although no adverse effects developed.

Small Plots.

These plots consisted of four rows of beets 35 feet long. The seeds were treated with American Cyanamid 12008 or 3911 in three ways: (A) in 50-percent impregnated carbon dust, (B) in emulsions at the rate of one gallon to 15 pounds of seed, and (C) in an emulsion spray directed into the seed row before the seeds were covered. Special equipment was con-

structed for the last treatment by mounting a spray nozzle directly behind the planting shoe of the planter. The rate of application was set at 13 gallons per acre and the desired quantity of the active ingredient was then diluted with water to 13 gallons.

Each treatment was replicated three times and its effectiveness was determined by caging leafhoppers on plants in the two-leaf and four-leaf stages of development, or 10 and 18 days after emergence of seedlings. In the two-leaf stage glass-vial clip cages were attached to the leaves of four plants on each plot and five beet leafhoppers placed in each cage to make a total of 60 leafhoppers per treatment. When the plants reached the four-leaf stage, cylindrical cloth-covered cages 3½ by 7 inches were used, each cage enclosing two or three plants. Three of these cages were placed on each plot and 25 leafhoppers were placed in each cage to make a total of approximately 225 leafhoppers per treatment. After the cages had remained on the plants for 48 hours, they were removed and the leafhopper mortality determined.

Table 1.—Percent Mortality of Beet Leafhoppers in Cages on Small Plots of Various Seed Treatments.

Insecticide (American Cyanamid Number)	Pounds of Active Ingredient per Acre (15 Pounds of Seed)	On Two-Leaf Plants	On Four-Leaf Plants
	Carbon Dust on Seed		
12008	¼	55	25
	½	91	33
3911	¼	66	28
	½	83	47
	Emulsion on Seed		
12008	¼	47	40
	½	52	16
3911	¼	38	36
	½	62	53
	Emulsion Spray in Seed Row		
12008	1	43	24
	2	85	39
3911	1	51	28
	2	81	18
Check (no treatment)			

The results are given in Table 1. At the two-leaf stage, some mortality was indicated for most of the treatments. Seed treated with the 50-percent carbon dusts produced better results than did seed treated with the emulsions. Good results were also indicated from spraying directly into the seed row, but two pounds of the active ingredient were required to produce about the same results as were obtained with ½ pound when applied in carbon dust on the seed. By the time the plants had reached the four-leaf stage, considerable toxicity to the leafhoppers was lost, but there were still some mortalities due to treatment. These data indicate that, after the two-leaf stage, the effectiveness of the treatment rapidly declines.

Large Plots.

Two five-acre plots of seed beets were planted with treated seed on a commercial field. A beet variety resistant to curly top was chosen, so that leafhopper populations could be observed on both treated and untreated

Table 2.—Numbers of Beet Leafhoppers per Foot of Row on Five-Acre Plots in Different Stages of Development in Which Seeds Were Treated With American Cyanamid 12008.

Treatment	Pounds of Active Ingredient per Acre	Cotyledon (6)[1]	Two-Leaf (8)[1]	Four-Leaf (14)[1]	Six-Leaf (21)[1]	50-75 Percent Foliage Coverage (29)[1]
Emulsion	¼	0.04	0.00	0.44	0.28	0.36
Carbon dust	½	0.02	0.02	0.26	0.20	0.32
Untreated check	—	0.70	0.64	1.06	1.48	1.12

[1] Numbers in parenthesis indicate days after emergence of seedlings.

portions of the field without the complication of commercial control with DDT. On one plot, the seeds were treated with an emulsion of American Cyanamid 12008 at the rate of ¼ pound per acre (15 pounds of seed) and on the other plot with 12008 in an impregnated carbon dust at ½ pound per acre. The numbers of leafhoppers per foot of row as determined with a counting cage are given in Table 2. Good results were obtained with both treatments. In fact, populations throughout the season on the plots grown from treated seed were well below what might be considered an economic level, whereas on that portion of the field grown from untreated seed they were so high that the field would have been dusted for leafhopper control had it not been a curly-top resistant variety.

Discussion

Although this paper reports only one season's work, the data indicate that it is possible to obtain control of the beet leafhopper on seedling beets by treating the seed before planting. They further indicate that beet plants grown from treated seed may remain sufficiently toxic to the leafhopper to protect them through the four-leaf stage, which in the Salt River Valley would be about two weeks after the seedlings emerge. It is probable that in many instances no further control would be needed.

Observations on both large and small plots indicate that seed treatment with either American Cyanamid 12008 or 3911 gives some protection against lepidopterous larvae in plants in the cotyledon and two-leaf stages. Heavily infested fields would probably have to be dusted once later, but protection to the small plants shortly after emergence could prevent the loss of stands.

Summary

Greenhouse tests with several systemic insecticides showed them to be toxic to the beet leafhopper, both as a soil drench and as a seed treatment. Field tests in small plots and in five-acre commercial plantings showed that seed treatment with the phosphorous compounds American Cyanamid 12008 and 3911 would protect the seedling plants for at least two weeks after their emergence.

References

(1) HILLS, ORIN A., MAST, A. A., and WOOD, R. C. 1948. Field insecticide tests against the beet leafhopper in sugar beets grown for seed. Amer. Soc. Sugar Beet Tech. Proc. pp. 298-318.

(2) ROMNEY, VAN E. 1943. The beet leafhopper and its control on beets grown for seed in Arizona and New Mexico. U. S. Dept. Agr. Tech. Bul. 855, 24 pp.

Isolation and Properties of Sugar Beet Araban

ALAN E. GOODBAN AND HARRY S. OWENS[1]

For more than 80 years it has been known that sugar beet pulp contains a gum which may be hydrolyzed to give a high proportion of arabinose (1)[2]. Later work has shown that this araban is a relatively low molecular weight polysaccharide, soluble in water. Its importance to the sugar beet industry is twofold; first, it is water soluble and can be extracted during the diffusion process and interferes with purification and crystallization of sucrose; and second, it composes from 15 percent to 20 percent of the marc solids and may be a useful by-product of sucrose production. Beet molasses contains an alcohol-insoluble polysaccharide which has been shown to have a very marked depressant effect on the rate of crystallization of sucrose (2). This material yields, upon hydrolysis, arabinose, galactose, dextrose, and mannose. Ingleman (3) also found that the alcohol insolubles from sugar beet molasses were inhibitors of sucrose crystallization. Since the original discovery of araban in the pulp was made by extracting the beets with lime water, it is apparent that conventional purification methods will not remove araban but allow it to be carried along to the crystallizers.

Extraction

We were interested in the extraction of araban from the standpoint of both the conditions necessary to give a good yield of representative material, and also the conditions which might be encountered in the factory which would allow araban to be extracted into the diffusion juice. The pulp used was a supply of factory cossettes which had been preserved in boiling isopropyl alcohol. The cossettes were ground and extracted with boiling 70 percent alcohol until the extract contained less than 1 percent sucrose. No arabinose was detected by paper chromatography of the hydrolyzed extract.

To determine the total amount of arabinose-containing polysaccharide in the pulp, portions were refluxed with 1N or 3N HCl for periods of 30 minutes to 24 hours. The extracts were analyzed chromatographically and the arabinose estimated by reflectance of the spot after development with aniline trichloracetate (4). The maximum arabinose was about 20 percent, obtained in 1 hour with 3N or 4 hours with 1N acid.

Extraction of the pulp was investigated by heating the pulp with 50 volumes of extractant, neutralizing the filtrate, and concentrating before hydrolysis. Arabinose was determined on the hydrolyzate. Extraction was for 40 or 120 minutes at pH 6, pH 9, or pH 11, and a temperature of 70° or 100° C. High-temperature electrodes were used to measure the pH during extraction, and 1N NaOH was added to maintain the desired pH. At pH 9 or pH 11, the de-esterification and degradation of pectin occur very rapidly and it is necessary to add alkali to neutralize the acid produced. The results of the extractions are shown in Table 1. Extraction at pH 6 is slight in comparison to the higher pH extractions, and 100° is more effective than 70°. Apparently the extraction of araban is correlated with the breakdown of the pectin in the cell wall, since pH 6 extraction also

[1] Chemists, Western Utilization Research Branch, Agricultural Research Service, U. S. Department of Agriculture, Albany 10, California.

[2] Numbers in parentheses refer to literature cited.

Table 1.—Extraction of Araban from Sugar Beet Pulp.

pH	Temperature ° C	Time Min.	Yield of Arabinose Percent on Pulp
6	70	40	.63
		120	.64
	100	40	1.1
		120	1.9
9	70	40	3.3
		120	4.3
	100	40	10.8
		120	12.0
11	100	40	19.3

fails to remove pectin. It is of interest to note that the pulp becomes slimy and difficult to filter when extracted at pH 9. This might be an explanation for the observed deterioration of pulp at high battery supply water temperatures. The battery supply water is usually alkaline because of condensate return, and this, coupled with the higher temperature, causes the degradation of pectin and the solution of araban. From these results it would seem desirable to control the alkalinity of the battery supply water to improve the pressability of the pulp and to minimize the extraction of araban.

Extractions also have been made with saturated lime water at 100°. The arabinose obtained was 13.5 percent in 40 minutes, and 18.5 percent in two hours. Since this is close to the total arabinose in the pulp, lime water was chosen as the extracting medium because of simplified control of conditions, ease of removal of excess calcium, and because the pectic acids produced form insoluble lime salts.

Purification and Isolation

The preparation of araban was undertaken in order to study the effect of this material on purification and crystallization studies in our processing laboratory. For our purposes it was desirable to obtain pure araban, or the purest arabinose-containing polysaccharide possible, in order to isolate the effects due to araban from those of the other colloidal materials in the beet.

Although the presence of a high proportion of arabinose in a gum from sugar beet pulp was known as early as 1873 (1), the first report of the isolation of an araban from sugar beets was by Gaponenkov (5) in 1936. This material was obtained by extraction of hydropectin by 70 percent ethanol and yielded 98 percent arabinose on hydrolysis. It is claimed by Schneider and Bock (6) that this extraction procedure, which has been used by other workers, actually extracts only the lower-molecular-weight arabans, and indeed the material isolated by Gaponenkov was fractionated by precipitation with 80 percent, 75 percent, and 70 percent alcohol into fractions with molecular weights of 5,700, 6,600, and 7,700 respectively. In 1945 Ingelman (3) purified an araban from sugar beets by dialysis and electrophoresis and determined the molecular weight to be about 10,000, and the specific rotation to be —129°. Hirst and Jones (7) have reported on the structure of sugar beet araban. It is a highly branched chain of L-arabo-furanoside residues, $\frac{2}{3}$ of the units linked through positions 1 and 5, the remainder being terminal groups linked through positions 1 and 3.

We have found that extraction of pulp with lime water and precipitation of araban with alcohol yields an impure araban, as noted by Hirst and Jones. We have resorted to fractionation of the acetylated araban followed by de-acetylation to give a purified araban.

In a typical isolation, 120 grams of sugar-free beet pulp was extracted for two hours at 100° in 1500 ml. of saturated lime water. The extract was treated with CO_2 to pH 8, filtered, and the residual ions removed with ion exchange resins. After concentration to 200 ml., araban was precipitated with 9 volumes of 95 percent EtOH, washed, and dried. Yield was 17.5 grams of araban; 14.9 grams of this crude araban was acetylated by the method of Carson and Maclay (8), using formamide as a dispersing agent. The product was precipitated in ice water, washed, and dried. The yield was 23 grams of almost white material, anhydroarabinose 48 percent, calc. for araban diacetate 60.2 percent.

Crude araban acetate prepared in this manner was fractionated by two methods, column fractionation on charcoal or fractional precipitation from acetone by the addition of petroleum ether. Details of the fractionations are being published elsewhere (9). Columnar fractionation was somewhat superior to the fractional precipitation.

Table 2.—Araban Recovered from Fractions of Acetate on a Charcoal Column.

	Araban Acetate		
Fraction	**(α)D**	**Purity Percent**	**Araban (α)D**
6	—56[1]	68[2]	...
11	—116	92	—131[3]
20	—84	...	—99
Original	—95	80	—113

[1] About 0.5 percent in chloroform.
[2] Calculated from arabinose content by orcinol-ferric chloride determination.
[3] About 0.5 percent in H_2O.

To recover araban from the acetate, samples were treated with 1N KOH for several days at room temperature until solution was complete. KOH was neutralized with excess acetic acid, then 8 volumes of EtOH were added. The precipitated araban was reprecipitated from aqueous solution to remove KOAc and dried. The yield was essentially quantitative. Data on fractions from a charcoal column are given in Table 2. The specific rotation is the best measure of the purity of the fractions. Fractions 6 and 11 were obtained by elution of the column with acetone-chloroform mixtures, fraction 20 by elution of the column with dioxane. The purities reported were calculated from the pentose values by the orcinol-ferric chloride method (10), which has consistently given results about 5 percent to 10 percent higher than paper chromatographic determination. A second fractionation, starting with an 89 percent araban acetate, gave fractions with a maximum purity of 95 percent, and a specific rotation of —122°. This material has not been deacetylated. Although even the best araban from these fractions still shows evidence of the presence of galactose and possibly a trace of rhamnose, it is probably suitable for use in studying the effect of araban on processing characteristics of sugar beet juices.

It is probable that the arabinose in sugar beet pulp is present largely as a true araban, since the yield of araban acetate with a purity of greater

than 90 percent was about 35 grams from 75 grams of starting material. The possibility of the presence of an arabo-galactan is certainly not excluded by these results, however, for none of the preparations were completely free of galactose, which is the principal impurity in the various materials prepared in this study.

Extraction of Commercial Grade Araban

There is another aspect to the araban in sugar beet pulp; namely, its value as a by product. Although araban from sugar beets is low in molecular weight, it may be useful, just as are other plant gums and mucilages, as an adhesive, a dispersing agent for emulsions, as a suspending agent in lotions, creams, etc. The drawback to the use of araban from sugar beet pulp is the expense of the usual alcohol precipitation. If $Ca(OH)_2$ is used as the extracting medium, the pectin is degraded to pectic acids which form insoluble calcium salts and can be separated by filtration. The excess calcium may be removed, along with the proteins, by carbonation in the usual manner. Araban can then be obtained by evaporation of the filtrate. This procedure has been followed in this laboratory to obtain crude araban as a light-brown powder. The use of carbon may be necessary to produce a light-colored product. The cost of this operation would be very low, since existing equipment in the factory could be used for almost the entire process.

Summary

The isolation of araban from sugar beet pulp has been described, and the conditions under which araban is extracted from the pulp have been investigated. The use of araban as a by product of the production of sugar is discussed, and an economical method for its preparation is outlined.

References

(1) SCHEIBLER. 1873. Ueber das Vorkommen der Arabinsaure (Gummi) in den Zuckerruben und uber den Arabinzucker (Gummizucker). Ber. 6:612.

(2) HARRIS, W. A., NORMAN, L. W., TURNER, J. H., HANEY, H. F., and COTTON, R. H. 1952. Methanol-insoluble material in sorgo and beet juices. Ind. Eng. Chem. 44:2414.

(3) INGELMAN, B. 1945. Results of sugar research in Uppsala. Socker Handl. 1: 179 (C.A. 39: 5525 1945).

(4) MCCREADY, R. M. and MCCOMB, E. A. 1954. Quantitative determination of sugars on paper chromatograms by a reflectance method. Anal. Chem. 26: 1645.

(5) GAPONENKOV, T. K. 1936. Physicochemical properties of araban. Colloid J. (USSR) 2: 561, (C.A. 31: 1649 1937).

(6) SCHNEIDER, G. G. and BOCK, H. 1937. Uber die Konstitution der Pectinstoffe. Ber. 70: 1617.

(7) HIRST, E. L. and JONES, J. K. N. 1948. Pectic substances pt. VIII. The araban component of sugar beet pectin. J. Chem. Soc. 1948: 2311.

(8) CARSON, J. F. and MACLAY, W. D. 1946. Acylation of polyuronides with formamide as a dispersing agent. J.A.C.S. 68:1015.

(9) GOODBAN, A. E. and OWENS, H. S. Chromatographic fractionation of sugar beet araban. Manuscript in preparation.

(10) FORNELL, W. R. and KING, H. K. 1953. The simultaneous determination of pentose and hexose in mixtures of sugars. Analyst 78:80.

Inheritance of Raffinose Production in the Sugar Beet

R. R. Wood, R. K. Oldemeyer and H. L. Bush[1]

Introduction

The sugar beet is known to contain many substances other than the sucrose which is commonly recovered as a commercial product. In general these substances tend to be melassigenic in the process of sucrose recovery from the beet. Usually, in factory operations these currently unwanted chemical compounds are referred to by the one term "impurities," regardless of their individual nature. It seems, however, that in order to make the most effective study of such a problem it might be well to separate these impurities into component parts if the methods are available to effect such a separation.

One of the principal carbonaceous compounds in this general classification is the tri-saccharide, raffinose, with the chemical make-up $C_{18}H_{32}O_{16}$. At the present time no commercial use is made of this sugar and the total United States consumption is about 100 pounds yearly for laboratory use. Consequently, it would be desirable to eliminate or reduce this nuisance from the sugar beet unless some further use of it can be found.

The study of the inheritance of raffinose production in the beet was made possible; first, by the development of a rapid chromatographic method for raffinose determination by Brown (1)[2] in 1952; second, the development by Powers (2) of biometrical methods for study of quantitative characters; and third, by the discovery of inbred lines of sugar beets in the "Cooperative Inbred Indexing Program" (3) having extremes of raffinose content.

Progeny test results from one mass selection for both high and low raffinose content of beets have been reported by Wood (4).

Materials and Methods

The biometrical analysis of the data given here follows rather closely the methods developed and/or described by Powers (2). Chemical analysis followed the method of Brown (1) with slight modification for large scale laboratory operation. Two inbred lines from the Inbred Indexing Program (3) were selected as parental material. These were (a) number 50-415 developed by the late G. W. Deming of the USDA and (b) number 410701 from H. L. Kohls at Michigan State University. Hybrids in the F_1 generation were identified by the use of hypocotyl color as a marker gene. Backcross generations were produced by similar technique. Six populations were studied; high raffinose parent P_1 (410701), low raffinose parent P_2 (50-415) F_1, F_2, B_1P_1, and B_1P_2. All the above six populations were grown in a randomized complete block experiment with twenty replicates for each population. Twelve, fully competitive (12-inch by 22-inch spacing) beets were taken consecutively from the center row of each three-row plot and analyzed individually; thus, a total of 240 roots constituted each population studied.

---

[1] Agronomist, Plant Breeder, and Statistician-Agronomist, respectively, The Great Western Sugar Company, Agricultural Experiment Station, Longmont, Colorado.

The writers wish to express appreciation to Dr. LeRoy Powers for generous advice on the analysis of the data used in this report.

[2] Numbers in parentheses refer to literature cited.

Determination of raffinose is given as percent on dry substance in the beet juice and assumes a constant marc. Calculations are on obtained data without transformation.

Variability of Population

The variances of the parental and F_1 populations may be used to measure environmental variability if the parents are homozygous for the genetic factors under consideration. The total variance of the segregating generations is the sum of the genetic and environmental variability. In this study, the variances provide an estimate of the homozygosity of the parents. Inasmuch as the mean squares of the two parents and the F_1 (Table 1) do not differ significantly as measured by the standard errors of the variance as described by Fisher (5), it may be assumed that the parents are homozygous for genes conditioning raffinose production.

Table 1.—Variances and Their Standard Error for Percent Raffinose on Dry Substance for Six Populations Derived from Two Inbred Lines of Sugar Beets.

Population	Variance (Mean square)
410701 (P_1)	.120364 ± .00470
B_1 to 410701	.133624 ± .00579
F_1	.111687 ± .00405
F_2	.251375 ± .02050
B_1 to 50-415	.163038 ± .00862
50-415 (P_2)	.122431 ± .00486

Population Means

The obtained means of the various populations differ significantly in all comparisons except F_1 and F_2 as shown in Table 2. The means of the F_1 and F_2 populations are statistically equal and are equal to the average of the means of the parental populations. The means of the backcross populations are also equal to the average of the means of the F_1 and their respective parents. These relationships can exist only in the absence of measurable dominance and/or genic interactions or linkage. Comparison of obtained population means with calculated theoretical means on the arithmetic scale gives a good fit by both standard error and X^2 tests. The X^2 value for comparison obtained with geometric means eliminates the possibility that gene action in this case can be following the geometric scale. The scale thus can be considered as additive and not multiplicative.

Table 2.—Obtained Arithmetic and Theoretical Arithmetic Means Compared with Geometric Means for Percent Raffinose in Sugar Beets Based on Dry Substance.

Population	Obtained Arithmetic	Theoretical Arithmetic	Theoretical Geometric
50-145 (P_2)	0.58 ± .036	----------	----
B_1 to P_2	0.76 ± .038	0.86 ± .025	0.81
F_1	1.13 ± .034	1.06 ± .025	0.94
F_2	1.16 ± .051	1.09 ± .028	1.03
B_1 to P_1	1.25 ± .041	1.34 ± .025	1.31
410701 (P_1)	1.53 ± .036	----------	----

$X^2 = .273$ with P = between .95 and .98 for comparison between obtained and theoretical means.

Table 3.—Frequency Distributions as Percent for Six Populations for Percent Raffinose on Dry Substance.

	Upper Limit of Class														
Population	**.24**	**.39**	**.54**	**.69**	**.84**	**.99**	**1.14**	**1.29**	**1.44**	**1.59**	**1.74**	**1.89**	**2.04**	**2.19**	**2.34**
410701 (P_1)					.42	.83	6.67	10.83	18.33	17.92	20.00	18.33	2.92	2.50	1.25
B_1 to P_1		.42	.42	2.92	4.17	8.33	21.67	16.25	17.08	13.33	9.17	5.42	.42	.42	
F_1		.42	.83	2.92	6.67	17.92	24.16	19.58	14.58	7.08	3.75	1.67	.42		
F_2		.42	2.08	6.67	8.75	13.33	22.50	9.17	14.17	8.33	6.25	7.50	.42	.42	
B_1 to P_2	.83	5.00	15.00	16.67	25.83	17.50	12.92	2.92	1.25		.83	1.25			
50-415 (P_2)	11.25	12.92	21.67	17.08	21.25	9.17	3.33	2.50	.83						

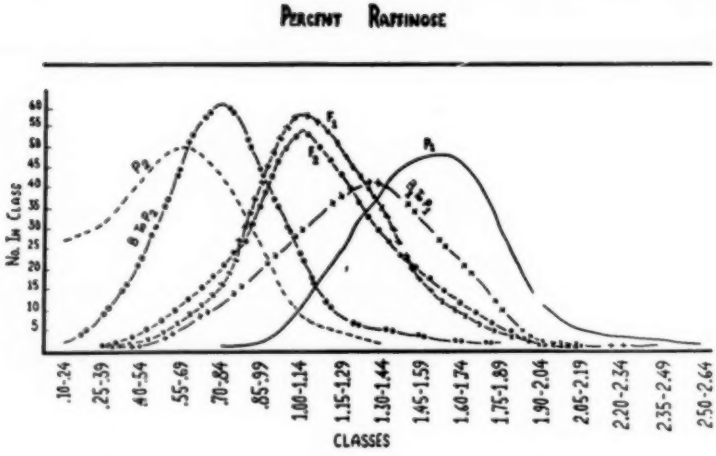


Figure 1.—Frequency distribution for six populations.

Genetic Analysis

Both Table 3 and Figure 1 indicate that the frequency distributions for the P_2 and B_1 to P_2 populations do not reach zero on the scale, indicating P_2 carries some genes for raffinose production.

Theoretical values for the ratios of the extreme genotypes each population has in common are given in Table 4. This table has been calculated from the expansion of the binomial for the backcross and F_2 populations, as demonstrated by Powers (2); the ratios are developed assuming no dominance, linkage, or gene interactions; and that effective factor pairs for the production of raffinose are equal in magnitude, and that one effective factor pair is non-isodirectional (or in other words, one effective factor pair for raffinose production enters the cross from the low raffinose parent).

One premise in the use of genotypic ratios for determination of effective factor pairs is that the frequency distributions should not be inhibited at the end of the curve from which the ratios are determined. It was indicated above (Table 3 and Figure 1) that both the P_2 and to a lesser extent the B_1 to P_2 populations, appear to be inhibited at the lower end of the population curve. The lower classes of the P_2 and B_1P_2, however, are probably accumulated rather than inhibited. In the chemical analysis used it is necessary to accumulate numbers in the lower classes due to the lack of refinement in measuring minimal values. Since arithmetic accumulation is involved in the determination of the ratios of the extremes of the populations, this analytical accumulation should not influence the obtained ratios unless there is accumulation beyond the classes which involve the genotypes under consideration. Assuming this to be an accumulation, rather than an inhibition, it is possible to utilize both extremes of the frequency distributions in estimating genotypic ratios.

Table 4.—Theoretical Genetic Model for Number of Factor Pairs. One Effective Factor Pair Non-isodirectional but All of Equal Magniture in Effect.

Number of Factor Pairs	Either B_1 to P_2 / F_1 or B_1 to P_1 / F_1	Either B_1 to P_1 / P_1 or B_1 to P_2 / P_2	Either F_2 / P_1 or F_2 / P_2	Either F_2 / B_1 to P_1 or F_2 / B_1 to P_2
2	75.000	75.000	68.750	125.000
3	50.000	50.000	34.375	87.500
4	31.250	31.250	14.453	56.250
5	18.750	18.750	5.469	34.374
6	10.9375	10.938	1.929	20.314
7	6.250	6.250	0.647	11.726

Table 5.—Ratios, as Percentages, Between Frequency Distribution Classes for Specified Populations Percent Raffinose on Dry Substance.

B_1 to P_1 / F_1	B_1 to P_2 / P_2	F_2 / P_2	F_2 / B_1 to P_2	B_1 to P_2 / F_1	B_1 to P_1 / P_1	F_2 / P_1	F_2 / B_1 to P_1
99.60	97.92	77.09	85.42	56.54	12.59	12.59	74.51
95.76	97.48	63.45	78.73	71.67	25.04	33.36	82.09
89.84	96.98	55.60	65.09	74.73	34.29	32.42	89.14
81.83	86.60	33.48	57.33	81.83	45.71	36.43	94.37
74.73	75.24	21.29	38.66	89.84	56.42	45.65	98.32
71.67	59.60	14.57	28.30	95.76	67.43	50.24	100.00
56.54	45.44	5.45	24.45	99.60	84.82	69.63	
	24.12		12.00		92.48	82.44	
	7.38		7.20		96.26	90.84	

Table 5A.—Indicated Number of Factor Pairs for Each of Eight Genotypic Ratios.

	B_1 to P_1 / F_1	B_1 to P_2 / P_2	F_2 / P_2	F_2 / B_1 to P_2	B_1 to P_2 / F_1	B_1 to P_1 / P_1	F_2 / P_1	F_2 / B_1 to P_1
Indicated No. of Factor Pairs	3	3-5	3-5	5-6	3	3-5	3-5	5-6

The obtained ratios for the various comparisons are listed in Table 5. The following number of factor differences between the two parents were estimated when the obtained values are compared to the theoretical values for different numbers of factor pairs (Table 4).

This information indicates that the two parents differ by about 5 effective factor pairs for raffinose production, one of which is non-isodirectional.

Inheritance in Relation to Results of Previous Mass Selection

It was reported by Wood (4) that progeny of one mass selection for low raffinose content had about 25 percent lower raffinose than the parent variety. This reduction is in line with the findings of this inheritance study. Assume the parental variety from which the selection was made had the same genetic characteristics as the F_2 population studied. 10 beets were

chosen from 150 in the selection. If these represented the lower 1/15 of the genotypic distribution, assuming 5 effective factor pairs of equal magnitude in effect, additive, and in equilibrium, the theoretical reduction in raffinose should have been 60 percent; the result of a reduction in gene frequency from 50 percent to 20 percent. However, with genetic and environmental variability confounded, it might be assumed that the roots selected had genotypes falling in the lower half of the genotypic distribution. On this basis the frequency in raffinose genes would be reduced from 50 percent to 37.7 percent, resulting in a reduction in raffinose of only 25 percent.

Summary

1. The inbred lines of sugar beets used as parental material for this study were apparently homozygous for the factors each carried for production of raffinose in the beet.

2. Quantitatively, the factors for raffinose production in the two parents followed an arithmetic scale and consequently are additive.

3. The number of effective factor pairs for production of raffinose between the two parents is about five and at least one is non-isodirectional and all are equal in magnitude.

4. Neither dominance, heterosis, nor linkage appeared to be involved in the cross studied.

References

(1) BROWN, R. J. 1952. Analytical Chemistry 24:384.

(2) POWERS, LEROY. 1955. Components of variance method and partitioning method of genetic analysis applied to weight per fruit of tomato hybrid and parental populations. Tech. Bulletin No. 1131, U. S. Dept. of Agriculture.

(3) BREWBAKER, H. E. and DOXTATOR, C. W. 1954. Cooperative testing of inbreds—three years results. Proc. Amer. Society Sugar Beet Tech. 8 (2): 75-78.

(4) WOOD, R. R. 1954. Breeding for improvement of processing characteristics of sugar beet varieties. Proc. Amer. Soc. Sugar Beet Tech. 8 (2): 125-133.

(5) FISHER, R. A. 1934. Statistical methods for research workers, p. 78. Oliver and Boyd, Edinburgh and London.

Effects of Sugar Beet Root Diffusates and Extracts, and Other Substances, on the Hatching of Eggs from the Cysts of the Sugar Beet Nematode, Heterodera Schachtii Schmidt

GERALD THORNE[1]

The fact that the roots of host plants secrete substances which diffuse through the soil and stimulate and attract their nematode parasites was suggested by Marcinowski as early as 1909 (3)[2]. As early as 1900, Buerkel (2) had found that a marine nematode, *Oncholaimus vulgaris* Bastian, was attracted to edible mussels used as "bait." Baunacke (1) conceived the idea that this fact might be used to stimulate the hatching of eggs from the cysts of the sugar beet nematode, and demonstrated that excretions secured by washing beet roots did increase the hatching of eggs. Following the work of Baunake, Rensch (4) attempted to isolate the stimulating substance and synthetically produced two compounds, A and B, which greatly increased the rate of hatching. Compound A was a component part of vegetable roots, whereas B contained a substance found in the soil when plant material is breaking down. Although Rensch stated that field demonstrations were planned, he did not report them, indicating either that they were not made, or that they proved unsatisfactory.

Studying the emergence of the sugar beet nematode larvae under actual field conditions, the writer (6) reported on soil samples from 21 fields which had been in crop rotations for 1 to 12 years. A total of 38,128 cysts was examined and the data obtained showed that, even though the common host plants were not present, there was a continual emergence each year, with the bulk of the populations hatched by the end of the sixth year. Emergence was especially heavy the first year when it was estimated that more than half of the larvae left the cysts.

The problem of host stimulation and the manner in which nematodes respond to root diffusates were well discussed by Steiner (5). Citing a number of instances, he came to the conclusion that plant infecting nemas not only have the ability to recognize host plants, but are able to distinguish the preferred one. He postulated that the active parts of root secretions are of a rather simple chemical nature and that the element or elements which stimulate the larvae to hatch from eggs within the cysts are not the same as those which direct them to the roots. These elements are carried in soil water and recognized by the nemas, after which they proceed to the preferred roots.

In the same paper, Steiner discussed the possible methods by which nematodes perceive these stimuli, offering the very plausible explanation that they are detected by means of the amphids, a pair of organs located on or near the head. By means of these organs "the nema is led to its pre-

[1] Senior Nematologist, Horticultural Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture, Salt Lake City, Utah.
[2] Numbers in parentheses refer to literature cited.

ferred host by some chemotaxic influence exerted by the plant." Apparently the young growing roots are the principal source of these secretions.

Wallace (9) found that 25° C. (77° F.) is the optimum temperature for hatching the larvae in the laboratory. But since field temperatures are not constant, he conducted a series of experiments in which temperature varied and found that 8 hours of 24° C. followed by 16 hours at 15° C. produced a marked increase in emergence. Other experiments demonstrated that low oxygen concentrations reduced emergence.

Procedures Followed in These Experiments

Experiments designed to study, the effects of root diffusates or other substances on the hatching of sugar beet nematodes should follow natural conditions as nearly as possible. The nature of the work demands that most of it be done in the laboratory and immediately we introduce a series of unnatural conditions which may produce results entirely different from those existing under field conditions.

This work was conducted at the Salt Lake City, Utah, station during the years 1934 through 1939[8]. It will be noted that in these experiments we digressed from the usual procedures followed by other workers on the sugar beet nematode, and by those investigating the golden nematode of potatoes, *Heterodera rostochiensis* Woll., a relative of the sugar beet nematode:

1. Soil and small roots from severely infected sugar beets were collected in late October and stored in a cool basement. Portions were removed as needed and washed by the Cobb sifting and gravity methods. The cysts were picked directly from the screen residues. This method is in direct contrast to procedures used by other workers who air-dry the soil, immerse it in water and skim the floating cysts off the top. Since only a very small portion of the soil in a field ever becomes air-dried by lying on the surface, it is obvious that such a method introduces a condition entirely foreign to the usual habitat of the nemas. This is especially true of cysts which most investigators not only air-dry, but store under refrigeration for weeks or months before using them.

2. Water was obtained from a canyon stream which carried in solution many of the elements found in field soil.

3. Coarse sand grains from the stream bed were placed in the hatching tubes to simulate further natural conditions.

4. Light was excluded for obvious reasons.

5. Small sugar beets were carefully dug with the least possible injury to the roots, rinsed, and placed in the tubes to stimulate hatching.

6. Extracts were prepared by crushing the roots of a beet, about ½ inch in diameter, in 100 ml. of water and filtering the resultant liquid.

7. Cysts were opened and their contents recorded when the experiments were completed.

---

[8] The assistance of Margaret Conder Deming in making many of the counts is gratefully acknowledged.

Selection of Brown Cysts for Experimental Purposes

Normally the female of *Heterodera schachtii* transforms to a brown cyst containing from 10 to more than 600 eggs in which the larvae are developed and become active if released. But in almost every lot, there were a few cysts in which the eggs were still in various stages of segmentation, even as late as mid-June. How much longer segmentation and larval development might have continued was not determined. Climatic conditions doubtless have considerable influence on larval development and these observations, under Utah conditions, may not preval in California and other states. In rare instances, the cysts did not contain viable eggs, perhaps because the female was not fertilized or the sugar beet was dug before the female was mature.

Cysts from beet dump dirt were not used because the heating and fermenting process during decay of the organic matter present, usually kill both larvae and eggs. Often cysts from this source are filled with fungi which thrive on the dead contents. Only rarely, if ever, are living larvae and eggs within the cyst attacked by fungi.

Cyst walls are very resistant to decay and they may remain in the soil for many years, accumulating in enormous numbers. Of the thousands present in a soil sample, only a few may have developed during the preceding season and contain a full complement of eggs. Partly, or entirely, empty cysts generally float during the washing process and are poured off and discarded. Selection of floating cysts for experimental purposes is therefore inadvisable.

Cysts for these experiments were selected from residues which passed through the 25-mesh per inch screen, and were collected on the 50-mesh, and settled to the bottom of the containers. These residues were thoroughly rinsed until clear, after which the cysts were picked out with a pair of sharp-pointed tweezers. When residues were properly prepared, it required only a few minutes to obtain a hundred cysts.

A minimum of 50 cysts was used, some experiments contained 500. Large numbers eliminate excessive errors from non-viable eggs. After the experiments were completed, cysts were opened and the contents examined. In some cysts every egg had hatched and the larvae emerged, while in a few none had hatched. In one cyst, 625 fully developed larvae were present which became active when the egg shells were ruptured. Occasional cysts contained two or three hundred hatched larvae which apparently had been unable to escape because there were no openings in the cyst walls. A few cysts contained dead eggs and larvae infected by fungi.

Saprophagous nematodes, *Acrobeloides butschii* (deMan), *Eucephalobus oxyuroides* (deMan) and other species, often inhabit the cysts and the beginner should not confuse them with sugar beet nematode larvae (7).

Resume of Experiments

Data are here presented for only two of the many experiments conducted. These are typical of the results obtained, and the conclusions drawn were verified by repeated trials.

Equipment: Six carbon filter tube funnels, 35 mm in diameter, were fitted with drain tubes and stop cocks. (Figure 1B). These were mounted on a galvanized iron frame 150 mm wide by 200 mm long and 175 mm high (Figure 1A). Glass tubes 25 mm in diameter and about 100 mm long were covered on one end with coarse-mesh bolting silk and served as containers for the cysts and sand. Mailing tubes fitted about the funnel excluded most of the light and box covering the entire assembly made it completely dark.

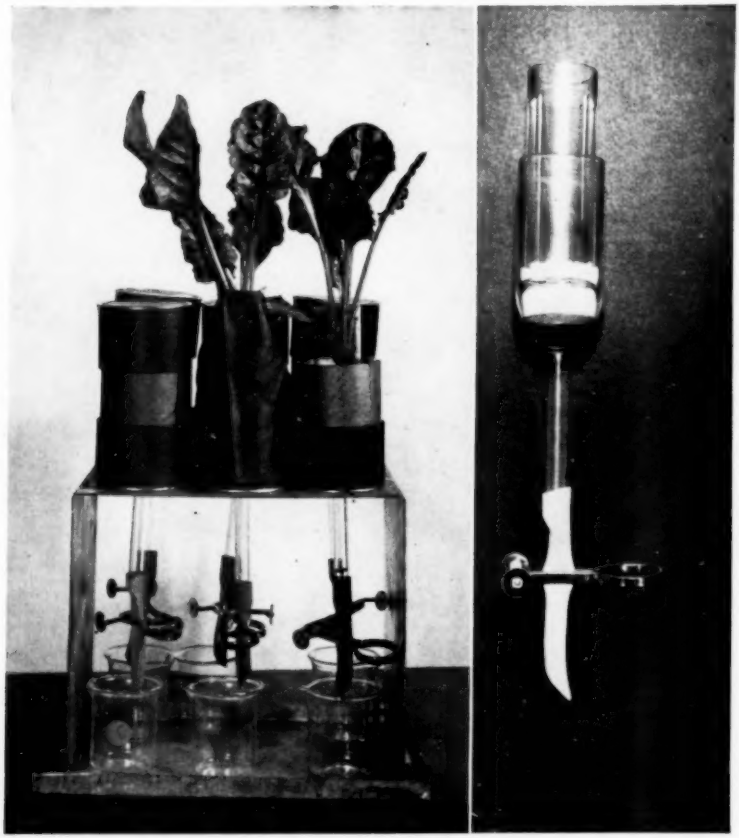


Figure 1-A (left). Frame holding funnels enclosed in mailing tubes, two small beets in place. 1-B (right). Funnel with tube in place.

Procedures: Cysts were placed in the tubes filled with the desired solutions. Each day, or at other specified intervals, about one-half of this solution was drawn off and immediately replaced by fresh solution poured into the tube in a small stream to insure aeration.

Cysts were examined by crushing on a cross-section microscope slide under a 25 mm square coverglass. Doubtless this crushing process released a few unhatched larvae from the eggs, but the numbers were negligible.

Larval counts were made under a low power binocular miscroscope. When numbers were near 500, two or more aliquots were selected, the larvae counted and the total was computed from these.

In the following described experiment, three lots of 500 cysts each, A, B, and C, were placed in canyon stream water on December 15. The first count of larvae was made December 18 and each day thereafter through the 27th. After the count was made on December 27, a young sugar beet was placed in A and extracts of young beet roots were added to C, while B continued in water only. During the following 10 days, the emergence was as recorded. These data indicate that young sugar beet roots stimulated hatching while the root extracts retarded it (Table 1).

Table 1.—Larvae Hatched from 3 Lots of 500 Cysts Each of *Heterodera Schachtii* Placed in Water from a Canyon Stream, December 15, 1934.

Date	A	B	C
Dec. 18	5,775	4,626	10,701
19	3,116	1,650	7,750
20	3,536	4,577	4,125
21	3,290	3,133	2,226
22	2,746	1,711	2,293
24	2,444	2,423	1,730
26	1,186	1,167	1,630
27	837	2,569	762
Sub-total	22,920	21,856	31,217
Dec. 29	365[1]	1,096	362[2]
31	865	675	283
Jan. 3	7,379[1]	1,668	348[2]
4	1,660	637	2,099
5	605	781	225
7	1,331[1]	1,127	1,209[2]
8	6,126	426	1,262
9	377	478	441
10	239	309	13
Sub-total	18,947	7,197	6,242
Grand Total	41,867	29,062	37,459

[1] Small sugar beet placed in tube when count was completed.
[2] Beet root extract added after count was made.

The important point revealed by this experiment is the fact that even in the presence of young sugar beet roots only about one-third of the larvae emerged from the cysts. This was determined by counting the larvae remaining in 50 cysts from each of the three lots after the experiment was concluded. When such a large portion of the larvae fail to respond to young beet roots, it seems as if there must be other factors which inhibit emergence.

Table 2.—Daily Hatching of Larvae from 50 Cysts of *Heterodera Schachtii* in Water and Solutions of Theelin.

Date	Water	Concentrations of Theelin		
		1/100,000,000	1/50,000,000	1/1,000,000
March 23	450	980	1,645	682
24	4,995	4,550	5,849	2,031
25	2,830	2,140	1,845	1,189
26	968	840	680	425
27	229	517	208	46
28	110	252	123	0
29	98	63	54	0
30	82	25	16	0
31	26	3	3	2
April 1	20	1	1	2
Total	9,808	9,377	10,424	4,377
Viable eggs and larvae remaining in cysts	1,716	2,608	2,148	7,242
Grand Total	11,524	11,985	12,572	11,619
Percent emergence	85.11	78.24	82.92	37.67

Since this experiment began December 15, the cysts were not sufficiently aged and a much greater emergence would have occurred had they been held 2 or 3 months longer. It appears obvious that the neophyte, testing materials for possible hatching stimulants, should not become excited if large numbers of larvae emerge during the first few days of an experiment.

Maturity of cysts had a decided influence on emergence of larvae. Four lots of 50 cysts each were placed in tubes on January 28, March 3, March 23, and May 18. At the end of 20 days the percentage of larvae hatched was 47.5, 69.4, 78.1, and 67.5, respectively. The low emergence from the May 18 lot was due to the fact that large numbers of the larvae had already hatched before the soil was washed to secure the cysts, many larvae being collected on the 200-mesh screen at that time.

Indol-3-acetic acid: A concentration of 1/50,000,000 of this chemical had a depressing effect on larval emergence; only 10.04 percent hatched as compared with 44.55 percent in the water check in early February.

Vitamin B.: When Vitamin B_1 was used in concentrations of 1/100,000,000, 1/50,000,000 and 1/1,000,000, hatches of 70.4, 77.9, and 86.1 percent, respectively, were secured as compared with 76.0 percent in water in early February.

Theelin: Results of the theelin experiments are shown in Table 2. Little, if any, stimulation was secured from the 1/100,000,000 and 1/50,000,000 concentrations, and 1/1,000,000 strength apparently acted as a depressant. Emergence in water in this experiment was the highest recorded in the many tests conducted.

Summary

Experiments to determine the effect of root diffusates or other compounds on the hatching of the eggs of *Heterodera schachtii* should simulate natural conditions as nearly as possible. Even in plain water a large per-

cent of the larva emerge and it is that portion remaining within the cyst at the end of the experiment toward which all efforts should be directed, because these are responsible for continuing the infestation during crop rotations.

References

(1) BAUNCKE, W. 1922. Untersuchungen zur biologie und bekampfung des Rubennematoden *Heterodera schachtii* Schmidt. Arb. Biol. Reichsanst. Land. u. Forstw. 11 (3) :185-288.

(2) BUERKEL, E. 1900. Biologische studien uber die fauna der kieler fohrde. 55. Kiel and Leipzig.

(3) MARCINOWSKI, K. 1909. parasitische und semiparasitisch an pflanzen lebende nematoden. Arb. K. Biol. Anst. Landw. Forstw. 7:1-192.

(4) RENSCH, B. 1924. Eine neue methode zur bekampfung der rubennematoden. Mitt. Deut. Landw. Gesell. 39:412-414.

(5) STEINER, G. 1925. The problem of host selection and host specialization of certain plant infesting nemas and its application in the study of nemic pests. Phytopath. 15 (9) :499-534.

(6) THORNE, GERALD. 1923. Length of the dormancy period of the sugarbeet nematode in Utah. U. S. Dept. Agr. Cir. No. 262.

(7) THORNE, GERALD. 1928. Nematodes inhabiting the cysts of the sugarbeet nematode, *Heterodera schachtii* Schmidt. Jour. Agr. Res. 37:571-575.

(8) WALLACE, H. R. 1955. Factors influencing the emergence of larvae from cysts of the beet eelworm, *Heterodera schachtii* Schmidt. Jour Helminth. 29 (1, 2) :3-16.

Determination of Saponin in Granulated Sugar: Method of H. G. Walker Adapted to Routine Control

ROBERT R. WEST and ROBERT S. GADDIE[1]

The analytical determination in commercial granulated sugar of trace quantities of substances loosely classified as "floc" which precipitate in acidified aqueous solution has become increasingly important the last three or four years. Before that time, spot checks were made in most sugar laboratories on the turbidity produced in sugar solutions by the addition of citric or phosphoric acid to a specified pH. Many adaptations to this general method have been tried. Various concentrations of sugar were used as were most of the common food and mineral acids at various pH's. Heating to different temperatures for varying periods of time as well as allowing the test to stand for periods ranging from one hour to ten days was specified. None of these modifications resulted in solutions from which quantitative measurements on floc-producing substances could be obtained.

As a result, it was impossible to predict in more than about 50 percent of the tests which sugars would tend to floc in susceptible beverages. Because the criterion on which the sugar is judged is the presence or absence of floc in these beverages, it seemed logical to base our search for a routine method of analysis on the behavior of sugar containing various concentrations of floc (as measured by the various methods of analysis) in one of the more susceptible carbonated beverages. The beverage chosen was 7-Up, not only because it is one of the most susceptible, but because it forms a clear water-white solution. We are indebted to Dr. B. C. Cole of the 7-Up product Department for furnishing the formula and flavoring ingredients for making 7-Up in the laboratory.

Cases of capped 7-Up bottles containing only carbonated water were obtained from the local 7-Up bottler. These were refrigerated to 0° C. The 7-Up syrup containing exact amounts of the various components including the sugar in question is prepared. The bottles of carbonated water are removed from the refrigerator and uncapped. Exactly 1 ounce of the water is poured off, exactly 1 ounce of the above syrup is added, and the bottles immediately recapped. At 0° C. practically no CO_2 is lost. Gas volumes on the recapped beverage varied from 3.7 to 4.1; 7-Up Company specifications are 3.5 to 4.0. After mixing, the bottles were allowed to stand at room temperature and observations for floc were made at intervals for periods of up to 8 weeks.

In 1952, F. G. Eis, et. al, published a method (1)[2] in which the floc from five pounds of sugar dissolved in five liters of water is precipitated in hot solution brought to pH 2.0 with HCl, filtered through a 600 ml. F-fritted glass funnel, extracted with hot methanol, the methanol evaporated and the triterpenes weighed. This method, while possessing a suitable degree

[1] Chief Chemist, General Laboratory, Utah-Idaho Sugar Company, Midvale, Utah, and General Chemist, Utah-Idaho Sugar Company, Salt Lake City, Utah, respectively.
[2] Numbers in parentheses refer to literature cited.

of quantitativeness, was too cumbersome for our purpose. The difficulties in filtering 5 pounds of sugar in 5 liters of water through a F-frit coupled with the small amount (6 to 10 mg. in the critical range for susceptible beverages) of recovered triterpenes rendered this method unsuitable for routine mass analysis.

Howard G. Walker, Jr., and Harry S. Owens, working on the acid-insoluble constituents in selected samples of sugar (2), found a fairly large percentage to be saponin and Mr. Walker devised a method for the determination of this component (3).

In the Walker method, the sugar is dissolved in water and the saponin precipitated by the addition of HCl. The solution is filtered through analytical Celite and the contents of the funnel dried. The saponin is then eluted with hot glacial acetic acid. To an aliquot of this eluate is added antimony pentachloride-chloroform reagent and the developed color measured by absorption at 535 mu.

We found that Walker's method could be readily adapted to routine mass testing of sugar and when evaluated by comparison of floc produced in 7-Up, gave consistent and reproducible results. This general method was, therefore, adopted and all values for saponin shown in this paper were obtained by our adaptation of this method. Table 1 shows typical results in 7-Up made with different sugars and mixtures of sugars from the seven Utah-Idaho factories.

Table 1.—Floc Production in 7-Up Made With Sugar Containing Various Concentrations of Saponin.

Appearance of Floc	Beverage Sample No.	Sugar—p.p.m. Saponin	Elapsed Time Days
Heavy, fine, dispersed	1	14.6	10
Large flakes	2	11.0	13
Heavy ring, deposit on bottle	3	8.0	17
Very fine, dispersed	4	7.5	13
Very fine, slight ring	5	6.0	20
Light film on surface	6	4.4	28
No floc	7	2.6	56
No floc	8	1.6	56

Three major conclusions were drawn from our work with the 7-Up.

1. Sugar saponin concentrations greater than 4 p.p.m. always resulted in detectable floc in the beverage if allowed to stand a suitable time.
2. The time required for the floc to become detectable is a function not only of the saponin concentration in the sugar but also of the source of the sugar.
3. The physical appearance of the floc varies rather widely.

In adapting Walker's method to the routine testing of sugar, we discovered very early that reagent grade antimony pentachloride contains free chlorine and must be purified before use. We attempted to remove this contaminant by adding crystalline antimony trichloride and by bubbling dry air through the reagent. Neither of these procedures was completely

effective on large batches (3 pounds) so vacuum distillation was necessary. As finally worked out, the distillation set-up consisted of all Pyrex glass assembly with standard taper connections throughout, a 2-liter distillation flask and a 1-liter receiving flask. The reagent is highly corrosive; also it decomposes in the presence of moisture. To exclude moisture and protect the laboratory vacuum pump we use a rather elaborate absorption train. Dr. Walker recommends using an all-glass aspirator with a freshly prepared $CaCL_2$ drying tube between the receiver and water pump.

Three pounds of reagent-grade $SbCl_5$ are placed in the 2-liter distillation flask along with approximately 10 gm. of Hengar boiling granules. The condenser should be full of water but the water should not be circulating. Pumping down time to an absolute pressure of 1 inch or less of mercury is approximately 2 hours at our laboratory because the rate at which the absorption train will take the gas evolved is rather low. As the vacuum reaches about 10 inches, evolution of gas (mostly chlorine) begins and continues without the application of heat for considerable time. When the absolute pressure reaches 1 inch Hg, heat is applied and the first vapors of $SbCl_5$ allowed to sweep the system. At this point water is immediately started through the condenser. The distillation rate is adjusted to give maximum recovery of reagent with minimum loss to the absorption train. This rate is such that about 1.25 hours are required and about 510 ml. of distillate recovered. By the time this much material has distilled over, the residue in the distillation flask is very dark and has developed a tendency toward unstable boiling. Another indication that distillation should be stopped is that the condensate in the connecting tube changes from a film to oily droplets.

The purified $SbCl_5$ is not stable under ordinary storage conditions and begins to darken in a matter of days. Therefore, the freshly distilled compound should be diluted immediately with chloroform, (12.5 percent $SbCl_5$ in $CHCl_3$, vol./vol.) This diluted reagent has been kept for periods up to six months with no significant deterioration when normal precautions were taken to exclude atmospheric moisture. In no case did the variation in absorbance during this period exceed 5 percent of the value of the 5 ppm. standard. As a result, the slope of the calibration curves remained nearly constant over the linear range (0-10 p.p.m. saponin) for a given batch of reagent. Successive batches of reagent prepared by the same technique gave deviations in absorbance values for saponin standards of $\pm$ 10 percent or less.

Saponin Standards

Saponin standards used in this work were obtained from different sources but were both extracted from diffusion juice by the method recommended by Walker (4). Sample "A" was extracted from concentrated diffusion juice (source unknown) and sample "B" was extracted from concentrated diffusion juice from one of the Utah-Idaho factories. Absorbance values for these two saponins made up in glacial acetic acid solution and read in an 18 mm. round tube at 535 mu are shown in Table 2.

Table 2.—Corrected Absorbancies on Saponin Standards.

Concentration	Sample A	Sample B
.05 mg./ml.	.250	.265
.10 mg./ml.	.500	.540
.15 mg./ml.	.610	.660

It may be seen that there is a slight difference in absorbance at the same concentration between these samples. It should be remembered, however, that the so-called "saponin" in each case is actually the alcohol-soluble fraction of the acid precipitate from diffusion juice. This fraction undoubtedly contains traces of other compounds which affect color development. We do know that the degree of purification of the saponin has a bearing on the color development. Saponin to be used for standards should be dissolved in alcohol and reprecipitated sufficient times to give a very light-tan-colored solid.

Analytical Method

To precipitate the floc producing substances, Walker (3) recommends the addition of a measured amount of concentrated HCl to the solution of the sugar under test. Eis, et. al. (1) acidify to pH 2.0, heat to boiling, and simmer for 10 minutes; Walker and Owens (2) recommend pH 1.0 to 2.5 for fairly rapid formation. We have found in working with granulated sugar that at room temperature and at a concentration of 50 gm. sugar in 150 ml. water, maximum recovery of saponin is not realized above pH 1.0 (see Table 3).

Table 3.—Recovery of Saponin at Various pH.

pH	Saponin, p.p.m.	Recovery, Percent of Maximum
1.0	11.2	Maximum (100%)
1.5	9.6	85
2.0	4.0	36
2.5	2.7	24
3.0	1.6	14

Reproducibility of Method

During the 1954-55 campaign, numerous samples of the same sugar from each factory were run on successive days. Sugars were chosen with floc contents ranging from 1 to 12 p.p.m. A summary of results is shown in Table 4.

Table 4.—Reproducibility of Method.

Factory	Sample No.	Average Floc p.p.m.	Maximum Deviation p.p.m.	Number of Analyses
A	1	10.0	± 0.3	8
A	2	1.6	± 0.1	12
B	1	1.8	± 0.1	12
C	1	1.4	± 0.1	8
D	1	2.0	± 0.2	8
E	1	8.0	± 0.3	12
E	2	12.0	± 0.4	12
F	1	11.6	± 0.5	16

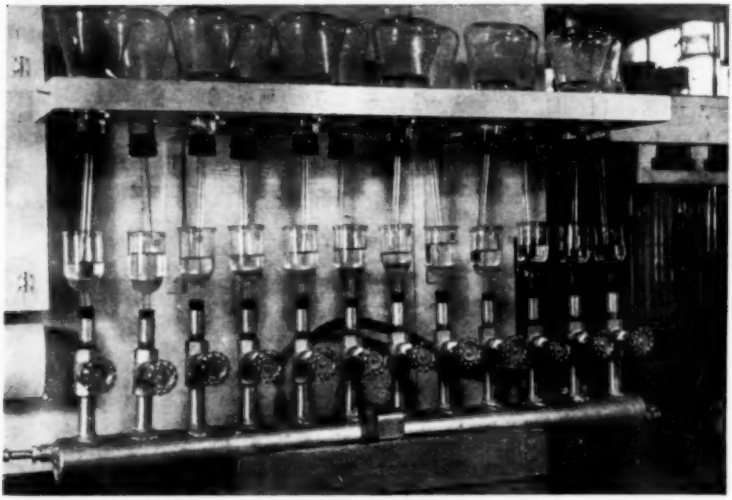
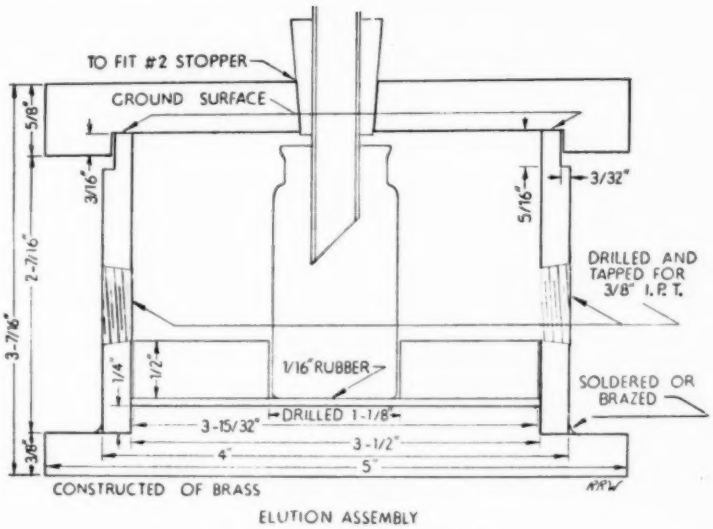


Figure 1.—Multiple filtration apparatus.

Method for Routine Analysis

In a 500 ml. Erlenmeyer flask dissolve 50.0 gm. sugar under test in 150 ml. distilled water at room temperature. Add concentrated HCl to pH 1.0 to 1.4 and mix. Rinse a clean "F" porosity 60 ml. high form fritted glass funnel with 5 ml. hot glacial acetic acid followed by 30 ml. water. Filter the sugar solution through this funnel. Multiple filtrations may be set up by means of an apparatus such as that shown in Figure 1. The unit consists of a vacuum header into which is welded 12 half-inch nipples. Attached to the nipples are half-inch valves to control the vacuum on each. Into each valve is screwed a short length of half-inch pipe squared and reamed at the end. The stem of each funnel is equipped with a No. 1 rubber stopper which just fits into the half-inch pipe. Automatic filtration is provided by a rack to carry the inverted 500 ml. Erlenmeyer flasks which are fitted with 1-hole stoppers into which are inserted short lengths of 10 mm. glass tubing.

When filtration is complete, rinse the flask with 15 ml. of very dilute HCl (1 ml. concentrated HCl + 1 liter of water), pouring rinsings into funnel; wash down sides and fritted surface of funnel using 15 ml. of the same very dilute HCl using gentle suction and several washings of the fritted disc. Dry the funnels for 1 hour at 103° to 105° C. and allow to cool. Greenish spots on the sides or fritted surface of the dry funnels indicate that all the sugar was not washed out. These spots will dissolve during elution causing off-colors and subsequent errors. Place a 5-dram vial in the elution apparatus shown in Figure 2. This elution apparatus is constructed of bronze. The seal between the cover and base is a ground fit, and with the aid of a little silicone stopcock grease, is air tight. There



ELUTION ASSEMBLY

Figure 2.—Elution assembly.

is a hole drilled in the lid which just fits the No. 1 stoppers on the funnels. A recess is drilled in the inside bottom of the base to position the 5-dram vial directly below the hole in the lid. Stopcocks on each side regulate the vacuum.

Place a funnel in the cover and assemble so that the funnel stem extends into the vial. Take up 5.2 ml. hot glacial acetic acid, (a 5 ml. glass hypodermic syringe with a 21-gauge needle is convenient). Elute the saponin by covering the fritted surface and then applying gentle suction. Repeat this several times until the 5.2 ml. of acid is used. Remove the vial and stopper immediately with the plastic stopper. Hold until all samples have been aluted. 5.2 ml. of acid will give very close to 5.0 ml. of eluate.

When a number of samples are ready to read, pipette 1.0 ml. (we use a 3 ml. hypodermic syringe) aliquots of each into clean 5-dram vials. In additional vials pipette 1.0 ml. of the elution acetic acid for a reagent blank and 1.0 ml. of saponin standard. Rinse the syringe twice each time with about 1 ml. of the solution to be pipetted. From a burette equipped with a teflon stocpcock, add 7.0 ml. antimony pentachloride-chloroform reagent. Stopper and mix by swirling; avoid splashing on the plastic stopper and do not expose to direct sunlight. At the end of 10 minutes read in the photometer at 535 mu. Read the reagent blank and saponin standard first. On regular graph paper with absorbance (optical density) as ordinates and p.p.m. saponin as abscissas, plot the scale reading of the reagent blank vs. 0 p.p.m. saponin. Plot the scale reading of the saponin standard vs. p.p.m. in the standard. A standard containing 0.05 mg. per ml. (50 p.p.m.) for

samples of low saponin concentration, or one of 0.10 or 0.15 mg. per ml. for samples of high saponin concentration is used. Connect the two points with a straight line. This is the calibration curve for this set of samples. The curve shifts slightly from day to day so it is redetermined each day. The scale reading on the sugar sample is for 10 gm. sugar so the p.p.m. saponin as read must be divided by 10.

The antimony pentachloride-chloroform reagent will react with practically everything we have tried except teflon and glass. Even silicone stopcock grease in a burette stopcock dissolves. We, therefore, use a teflon stoppered burette. Water will decompose the reagent liberating HCl and leaving a deposit of white antimony pentoxide which is insoluble in water. Therefore, all glassware which has come in contact with the reagent must be first rinsed in chloroform, then in concentrated HCl before finally washing in water. It is good practice to dry the vials and photometer tubes in the oven before using.

After a time the fritted funnels become partially plugged resulting in progressively longer time to effect filtration. By keeping the funnels immersed in dilute chromic acid solution when they are not in use, the life of a fritted funnel is greatly lengthened.

References

(1) EIS, F. G., CLARK, L. W., MCGINNIS, R. A., and ALSTON, P. W. 1952. Floc in carbonated beverages. Ind. & Eng. Chem. 44:2844.

(2) WALKER, HOWARD G. JR., and OWENS, HARRY S. 1953. Beet sugars: acid insoluble constituents in selected samples. Agr. and Food Chem. 1:450.

(3) WALKER, HOWARD G. JR. 1956. Determination of saponins in refined beet sugars. To be published, Journ. Amer. Soc. Sugar Beet Tech.

(4) WALKER, HOWARD G. JR. 1956. Private communication.

Materials and Methods Used in Producing Commercial Male-Sterile Hybrids

BION TOLMAN AND RONALD JOHNSON[1]

Introduction

During the lifetime of the American Society of Sugar Beet Technologists, many reports have been made dealing with male sterility, self fertility, Mendelian male sterility and self sterility, as these principles are related to sugar beet breeding (3) (4) (6) (7) (9)[2]. It is not the purpose of this paper to discuss technical aspects of the principles mentioned above, but to indicate how the various "breeding tools" have been adapted to the commercial production of sugar beet hybrids.

Sugar beet hybrids, made by use of a cytoplasmic male-sterile and a self-fertile inbred, were first reported by Owen in 1946 (5). In the years to follow, many new inbred lines were produced by utilizing the true self-fertile character, and as lines of promise were developed, male-sterile equivalents of these lines were produced by introducing the cytoplasmic male-sterility factor and then backcrossing three or four times to the inbred line.

From this type of a program, the inbred CT9 and its male-sterile equivalent were developed. Top crosses and true hybrids made with CT9 demonstrated, that under controlled conditions where the male sterile could be rogued carefully, hybrids could be produced that would yield up to 125 percent of the mass selected varieties in common use by the sugar beet industry (1).

In 1952, Owen reported a second type of male sterility called by him, Mendelian male sterility, because its inheritance was controlled entirely by genes in the germ plasm or cell nucleus, in contrast to the major influence of the cytoplasm in cytoplasmic male sterility (8). While the F_1 generation of a cytoplasmic male-sterile and a type O pollinating line was completely male sterile, the F_1 generation of a Mendelian male-sterile and a pollinating line was completely male fertile. Owen pointed out the significance of Mendelian male sterility in producing vigorous F_1 pollinators for use in the commercial production of hybrid varieties (9).

By 1952, the only apparent stumbling block in the way of producing cytoplasmic male-sterile hybrids on a commercial scale was the occurrence, in the CT9 male-sterile line, of an occasional pollen producer and more frequently, plants classed as semi-male steriles, which, under some conditions, might produce some pollen (7).

The seriousness of these semi-male-sterile plants was evaluated by both the Amalgamated and Utah-Idaho Sugar Company. Field hybrids were produced and the male sterile in one set of plots was carefully rogued, while in the other plot, no roguing was done. The hybrids from these two plots were then thoroughly tested in variety plots and found to be identical

---

[1] General Agricultural Superintendent and Research Assistant, respectively, Utah-Idaho Sugar Company, Salt Lake City, Utah.

[2] Numbers in parentheses refer to literature cited.

in performance (10). This demonstrated that the semi-male steriles in CT9 were not of commercial importance so long as Elite seed increases were rogued carefully to keep out any contaminants.

Hybrids were also produced to compare the value of keeping the pollinator and male sterile separate, as compared to blending in the pollinator as the commercial seed increase was made. Subsequent tests of these hybrids indicated, that where commercial top cross parents were used as pollinators, or where F_1 pollinators were used, there was no advantage in planting the male sterile and pollinator separate.

With this information as a background, the Utah-Idaho Sugar Company decided, in the Fall of 1953, to begin commercial production of multigerm hybrid sugar beet seed.

Monogerm Hybrid Seed Development

From the references already cited, it is evident that many of the basic principles essential to the production of sugar beet hybrids were fairly well developed by 1948 and that it had already been demonstrated, with multigerm inbred lines, that hybrids of superior yielding ability could be produced.

In the Fall of 1948, discovery of the monogerm inbred lines SLC 101 and SLC 107 was made by V. F. and Halen Savitsky (11). During the next six years, monogerm lines of increased curly-top resistance and improved seed characteristics were developed (2) (14). Self fertility and male sterility were principal breeding tools used in the development of basic breeding stocks for the production of monogerm hybrids (12). By 1954, inbred lines with improved yielding ability, high sucrose content, and curly-top resistance, higher than U. S. 33, had been released to the sugar beet industry (13).

In 1954, additional monogerm lines, derived by V. F. Savitsky from backcrossing the monogerm lines SLC 600 and SLC 175 to such curly-top varieties as U. S. 22/4 and U. S. 35/2, were released by the U.S.D.A. to the sugar beet industry. The ground was now laid for the commercial production of monogerm hybrid seed. Just two years after commercial production of multigerm hybrids began, the Utah-Idaho Sugar Company shifted its entire commercial seed acreage to the production of monogerm male-sterile hybrid seed.

Production of Elite Seed Stocks

Elite seed increases of cytoplasimc male steriles are made on isolated plots of about .5 acres in size. The following planting pattern is quite generally followed: 2 rows pollinator; 2 rows blank; 12 rows male sterile; 2 rows blank; 2 rows pollinator, etc. This plot is rogued carefully to eliminate pollen producers and as many as possible of the semi-male steriles. In cases where monogerm elite stocks are being reproduced, the plot is also carefully rogued for multigerm plants. All roguing is done as early as possible to prevent pollination from undesirable plants. Roguing is facilitated if plants in the male-sterile strips are thinned to singles in the early spring.

Stock Seed Increases

Stock seed increases are made on fields of from 5 to 10 acres in size. The general planting scheme, as used with Elite seed plots, is followed.

Stock seed fields are too large to make detailed roguing practical. Some roguing in monogerm stock seed increases may be done to eliminate stray multigerm plants. Contamination with occasional multigerm seed will possibly not be eliminated until multigerm seed is no longer being handled in any of the same equipment that is being used for monogerm seed.

As stock seed increases are being made, it is important that the male sterile, being produced for planting commercial seed acreage, has good vigor. This can, of course, be accomplished by producing an F_1 male sterile for commercial seed production. This can be illustrated by an actual example. In 1952, 610 MSmm x 610 mm was increased at Saint George. In 1953, 610 MS x 91 mm was increased in Saint George. In 1954 (610 MS x 91 mm) x 91 mm was increased at both Saint George and in Oregon. In the Fall of 1955, the monogerm stock seed increases were (610 MS x 91 mm x 91 mm) x 108 mm. The Elite seed plot in Saint George, planted in the Fall of 1955, was (610 MS x 91 mm x 91 mm) x 117 mm and, in the Fall of 1956, the stock seed plot will be planted using this MS and 108 mm as the pollinator. All of these monogerm inbreds, except SLC 117 mm, were developed by V. F. Savitsky, working in cooperation with the Beet Sugar Development Foundation and the Sugar Crops Section of the United States Department of Agriculture. The SLC 117 mm inbred line was developed by Owen.

Use of these respective lines so as to keep the male-sterile seed in the F_1 generation insures a vigourous male sterile for both stock seed and commercial seed acreage.

Pollinators for Hybrid Seed

In producing CT9 hybrids, two types of pollinators have been used: Commercial varieties as top-cross parents and F_1 pollinators produced by using the SLC 824 aa Mendelian Male sterile.

Figure 1.—Elite seed plot for production of F_1 pollinator. The 824 aa Mendelian male sterile pollinator strips on each side were 12-rows wide. The pollinator variety was a sugar selection of SLC 202. Approximately 60 percent of the plants were rogued from the 824 aa strip.

In producing monogerm hybrids we have used almost exclusively F_1 pollinators. Variety test results indicate that the F_1 824 aa x CT9 is one of the best we have used to date.

The production of F_1 pollinators is a rather tedious procedure because all the Mendelian male-sterile plants must be inspected for sterility while still in the bud stage and approximately 50 percent of the plants rogued out. However, we have found it practical to produce 500 to 600 pounds of F_1 pollinator in any one year (Figure 1).

In producing hybrid seed of either multigerm or monogerm, we have dyed the pollinator and then mixed it with the male sterile in the ratio of one pound of pollinator to twenty pounds of male sterile.

Practical Results of Hybrid Seed Program

Detailed data obtained from extensive field trials are included in another paper and will not be repeated here. However, a summary of the data pertaining to four of the CT9 hybrids is shown in Table 1.

The figures shown in Table 1 are averages of four CT9 hybrids as compared to 5 and 6 of what might be listed as leading commercial varieties. In each case several of the commercial varieties were used as top-cross pollinators in making the CT9 hybrids that were averaged for comparison. It will be noted that the CT9 hybrids gave an encouraging performance at all locations.

It was also interesting to note the performance of top-cross hybrids as compared to the commercial top-cross pollinator parent. These comparisons are given in Table 2.

Table 1.—Comparison of the Average Performance of Commercial Varieties With the Performance of CT9 Male-Sterile Hybrids.

Location and Comparison	Tons Per Acre	Sucrose Percentage	Gross Sugar Per Acre
Washington			
Average Commercial Variety[1]	19.46	15.56	6060
Average Male-Sterile Hybrid	20.72	15.69	6502
Idaho			
Average Commercial Variety	20.93	18.43	7714
Average Male-Sterile Hybrid	22.19	18.31	8126
Garland			
Average Commercial Variety	21.44	14.75	6324
Average Male-Sterile Hybrid	22.58	14.72	6648
So. Dakota			
Average Commercial Variety	17.86	15.05	5376
Average Male-Sterile Hybrid	19.80	15.02	5948
Nebraska			
Average Commercial Variety	17.64	15.00	5292
Average Male-Sterile Hybrid	18.28	15.06	5482
Combined Averages			
Commercial varieties	19.47	15.76	6153
Male-Sterile Hybrids	20.71	15.76	6541

[1] The six Commercial Varieties compared in these tests were:
In Idaho, Nebraska, South Dakota—U. S. 35/2; U. S. 22/3; SLC 202; G. W. 359; Klein E. and American No. 2.
In Utah and Washington—U. S. 35/2, U. S. 22/3, SLC 202, and Sugar Selection SLC 202.

Table 2.—Comparison of CT9 MS Top Crosses with the Pollinator Parent. Average of Results from Idaho, Nebraska, and South Dakota Variety Tests (Total, 32 Replications).

Top Cross Pollinators and CT9 MS Hybrids	Tons Beets Per Acre	Sucrose Percentage	Gross Sugar Per Acre
G.W. 359	19.52	16.14	6302
CT9 MS x G.W. 359	20.44	15.92	6508
CT9 MS x F_1 (824 aa x G.W. 359)	20.13	16.19	6518
American No. 2	17.74	16.44	5832
CT9 MS x American No. 2	20.14	16.16	6510
U. S. 35/2	17.50	16.52	5782
CT9 MS x U. S. 35/2	19.14	16.37	6266
Klein E	21.79	15.65	6820
CT9 MS x Klein E	21.93	15.63	6856
CT9 MS x F_1 (824 aa x Klein E)	19.14	16.00	6124
Average of Top Cross Polinator	19.14	16.19	6184
Average of CT9 Hybrids	20.41	16.02	6540

Table 3.—Comparison of 610 mm MS Top Crosses with the Pollinator Parent. Results from Shelley, Idaho, Test, 1955 (8 Replications).

Top Cross Pollinators and 610 Hybrids	Tons Beets Per Acre	Sucrose Percentage	Gross Sugar
G.W. 359	21.15	17.26	7302
610 MS x G.W. 359	21.50	17.20	7396
610 MS x F_1 (824 aa x G.W. 359)	19.82	17.79	7052
American No. 2	19.03	17.19	6542
610 MS x American No. 2	22.01	17.05	7506
U. S. 35/2 (824)	18.53	17.36	6434
610 MS x U. S. 35/2	20.62	17.29	7130
Klein E	23.10	16.90	7808
610 MS x Klein E	22.68	17.26	7830
610 MS x F_1 (824 aa x Klein E)	18.32	17.33	6350
Average to Top Cross Pollinators	20.45	17.18	7021
Average of 610 mm MS Hybrids	21.70	17.20	7466

It will be noted that in every case the hybrids were higher than the top-cross pollinators in yield, although the hybrid with Klein E was not significantly higher than the Klein E parent. The increase in tonnage was strikingly higher when high sugar top-cross parents were used as compared to when tonnage type varieties were used as the top-cross parent.

These same relationships were obtained when the monogerm 610 MS was used in hybrids with these same top-cross parents. These results are shown in Table 3. It should be noted that the hybrids with the monogerm 610 MS gave the same percentage increase over the top-cross pollinator parents as did the multigerm hybrids with CT9.

In both Table 2 and Table 3, data are listed for a three-way hybrid where SLC 824 aa Mendelian male sterile was used to produce an F_1 pollinator for the main purpose of increasing the sucrose percentage of the hybrid. A further summary of the effect of introducing a high sugar variety into the pollinator is shown in Table 4. It is evident from these data, that the use of SLC 824 aa to produce an F_1 pollinator did increase the

sucrose percentage, but it also decreased tonnage sufficiently to result in less gross sugar per acre. This does not mean that some combinations cannot be made where results would be more favorable, or that a different Mendelian male sterile might not react differently.

Table 4.—Comparison of Two-Way and Three-Way Hybrids Based on Results with G.W. 359 and Klein E Hybrids Planted at Four Locations.

Location of Test	Tons Beets		Sucrose Percentage		Gross Sugar	
	Two-Way Hybrids	Three-Way Hybrids	Two-Way Hybrids	Three-Way Hybrids	Two-Way Hybrids	Three-Way Hybrids
Shelley, Idaho						
8 Replications	21.93	22.69	17.77	18.41	7794	8354
8 Replications	21.50	19.82	17.20	17.79	7396	7052
8 Replications	23.58	20.26	17.81	18.31	8400	7420
8 Replications	22.68	18.32	17.33	17.26	7860	6324
Nebraska						
12 Replications	17.73	18.52	15.01	15.08	5328	5586
12 Replications	20.31	18.79	14.54	14.86	5906	5584
South Dakota						
12 Replications	21.64	19.19	14.98	15.08	6484	5788
12 Replications	21.90	18.37	14.53	14.85	6364	5456
Garland, Utah						
15 Replications	24.01	22.25	14.14	14.51	6790	6456
Combined Average of 95 Plots	21.70	19.80	15.92	16.24	6910	6432

Commercial Seed Acreage Planted in 1955

In the Fall of 1955, 588 acres of sugar beet seed were planted for the purpose of producing commercial monogerm hybrids. Based on monogerm seed yields obtained in 1954, this acreage should produce well in excess of 1,000,000 pounds of monogerm seed. This seed will be harvested in 1956 and will be put to commercial use in 1957 and 1958. No data are available on the performance of the exact hybrids being produced. However, we have already seen in comparing data in Tables 2 and 3 that, when the same pollinators were used, hybrids with CT9 MS and 610 MS were very similar. Data are available on the pollinators used for the 1955 seed plantings. The Monogerm male sterile used in the seed plantings was 91 MS rather than the 610 MS, for which data are available. However, from available data (1954 Government Report to Beet Sugar Development Foundation) the 91 MS averaged 103 percent of 610 MS in both yield and sucrose percentage. We can expect, therefore, that hybrids produced using 91 MS will, in general, be three percent better than hybrids produced using 610 MS.

Actual performance of the pollinators used in 1955 seed plantings of 610 MS hybrids and their calculated performance with 91 MS are shown in Table 5. These data indicate that monogerm hybrids, now planted for seed production, will exceed the present commercial variety in both tonnage and sucrose percentage and that their performance will be equal to the best CT9 hybrids which we have produced.

Table 5.—Comparison of U. S. 22H Commercial with Monogerm Hybrids Using 610 MS and Multigerm Pollinators Used in Commercial Seed Plantings in Fall of 1955 (Shelley Test, 8 Replications).

Variety and Hybrid	Tons Beets Per Acre	Sucrose Percentage	Gross Sugar Per Acre
U. S. 22H Commercial	19.09	17.43	6658
610 MS x F_1 (824 aa x CT9)	22.57	17.34	7817
610 MS x Sug. Sel. 202	21.77	17.23	7482
610 MS x U-I 114	21.50	17.20	7394
610 MS x F_1 (824 aa x U-I 114)	19.82	17.79	7018

Calculated Performance of Actual Monogerm Hybrids Planted for Seed Production in Fall of 1955 Based on Use of 91 MS Rather Than 610 MS[1]

Hybrid	Tons Beets Per Acre	Sucrose Percentage	Gross Sugar Per Acre
U. S. 22H Commercial	19.09	17.43	6658
91 MS x F_1 (824 aa x CT9)	23.25	17.86	8286
91 MS F_1 (824 aa x S.S. 202)	22.42	17.75	7931
91 MS x U-I 114	22.15	17.72	7838
91 MS x F_1 (824 aa x U-I 114)	20.41	18.32	7439

[1] Calculations shown above are based on the improved performance of 91 MS over 610 MS as shown by Government tests in 1954. These comparisons are shown below.

Comparison of 610 MS and 91 MS in Hybrid Combinations When 610 mm and 91 mm Lines Were Used to Pollinate a Common Multigerm Male Sterile—1954 Gov. Report.

Location	Tons Beets	610 mm Sucrose	610 mm Sugar	Beets	91 mm Sucrose	91 mm Sugar
Taylorsville, Utah	29.70	14.60	8672	32.90	14.93	9824
Twin Falls, Idaho	31.40	17.77	11160	30.90	18.18	11236
Brawley, California	24.90	14.33	7136	24.40	15.02	7330
Average	28.67	15.57	8928	29.40	16.04	9432
Percent Increase				103%	103%	106%

References

(1) MURPHY, ALBERT, RYSER, GEO. K., SMITH, C. H. and OWEN, F. V. 1950. Performance of three male-sterile sugar beet hybrids. Proc. Amer. Soc. Sug. Beet Tech. pp. 184-186.

(2) MURPHY, ALBERT, SAVITSKY, V. F. 1952. Breeding for resistance to curly top in hybrids with monogerm beets. Proc. Amer. Soc. Sug. Beet Tech. pp. 387-389.

(3) OWEN, F. V. 1942. Inheritance of cross and self sterility and self fertility in *beta vulgaris*. Jour. Agric. Res. 64 (12):679-698.

(4) OWEN, F. V. 1945. Cytoplasmic inherited male sterility in sugar beets. Jour. Agric. Res. 71:423-440.

(5) OWEN, F. V., MURPHY, ALBERT, RYSER, GEO. K. 1946. Inbred lines from curly-top resistant varieties of sugar beets. Proc. Amer. Soc. Sug. Beet Tech. pp. 246-252.

(6) OWEN, F. V. 1948. Utilization of male sterility in breeding superior varieties of sugar beets. Proc. Amer. Soc. Sug. Beet Tech. pp. 156-161.

(7) OWEN, F. V. 1950. The sugar beet breeder's problem of establishing male-sterile populations for hybridization purposes. Proc. Amer. Soc. of Sug. Beet Tech. pp. 191, 194.

(8) OWEN, F. V. 1952. Mendelian male sterility in sugar beets. Proc. Amer. Soc. Sug. Beets Tech. pp. 371-376.

(9) OWEN, F. V. 1954. Hybrid sugar beets made by utilizing both cytoplasim cand mendelian male sterility. Proc. Amer. Soc. Sugar Beet Tech. VIII (2) :64.

(10) RUSH, GEORGE E. 1952. The relative performance of some non-rogued male-sterile hybrids. Proc. Amer. Soc. Sug. Beet Tech. pp. 65-69.

(11) SAVITSKY, V. F. 1950. Monogerm sugar beets in the United States. Proc. Amer. Soc. Sug. Beet Tech. pp. 156-159.

(12) SAVITSKY, V. F. 1952. Methods and results of breeding work with monogerm beets. Proc. Amer. Soc. Sug. Beet Tech. pp. 344-359.

(13) SAVITSKY, V. F., RYSER, GEO. K. 1954. Sugar content in mono- and multigerm sugar-beet hybrids, carrying the gene *m* isolated from Michigan Hybrid 18 and the gene *m* from variety U. S. 22/3. Proc. Amer. Soc. Sug. Beet Tech. VIII (2) :23-28.

(14) SAVITSKY, V. F., MURPHY, ALBERT M. 1954. Study of inheritance for curly-top resistance in hybrids between Mono- and Multigerm beets. Proc. Amer. Soc. Sug. Beet Tech. VIII (2) :34-44.

Drying Temperatures and Storage Problems of Sugar Beet Seeds

CARL W. HALL[1]

Sugar beet seed for Michigan growers is grown in the West and shipped into Michigan for processing and planting. All of the two-million pounds of sugar beet seed for the eastern sugar beet producing area, which includes Michigan, is grown in California and Oregon. (1) During transportation and storage the moisture content of the seeds changes because of humidity and temperature variations. An increase in moisture content above eleven or twelve percent occurs in Michigan seed during some years, causing difficulty in decorticating the seed. The engineering properties of sugar beet seeds were determined to aid in solving the problem of excess moisture. All tests were run using sugar beet seeds of variety 216 x 226 from the 1953 harvest, initially at 13 to 14.5 percent (wet basis) moisture. Conventional air-oven methods were used for making the moisture determinations.

Equilibrium Moisture Content

Sugar beet seeds will go to a certain moisture content, known as the equilibrium moisture content, depending upon the temperature and relative humidity of the surrounding atmosphere. The equilibrium data can be used to determine the moisture content changes which will take place during transporting and storing. Each sample of seeds of approximately 10 grams was placed in a glass container in which the relative humidity was maintained by a saturated-salt solution. Salts were selected which maintained approximately the same relative humidity at the different test temperatures as follows:

Relative Humidity, Percent	Chemical (salt)	
90	$Ba\ Cl_2.2H_2O$	Barium chloride
75	$Na\ Cl$	Common salt
53	$Mn\ Cl_2.4H_2O$	Manganese chloride

The glass container for each sample was then placed in temperature-controlled wooden boxes which maintained 100°, 85°, 60°, and 40° F. ± 1° F. With four different temperatures and three different relative humidities, there were 12 samples in a test. The test was run three times. There was a maximum variation of the equilibrium values of approximately three-fourths percent moisture from those reported in Figure 1.

Germination of Sugar Beet Seeds

If heated air is to be used for drying sugar beet seeds, it is important to know the effect of the heated air on the germination. Tests were run with the seeds in a thermostatically-controlled oven at 100°, 110°, 120°, 130°, and 140° F., with exposure times of 1, 2, 3, 4, 5, and 6 hours at each of these temperatures. Duplicate tests were made for each time and

---

[1] Professor of Agricultural Engineering, Michigan State University, East Lansing, Michigan. Approved for publication by the Michigan Agricultural Experiment Station as Journal Article No. 1869, January, 1956.

[2] Numbers in parentheses refer to literature cited.

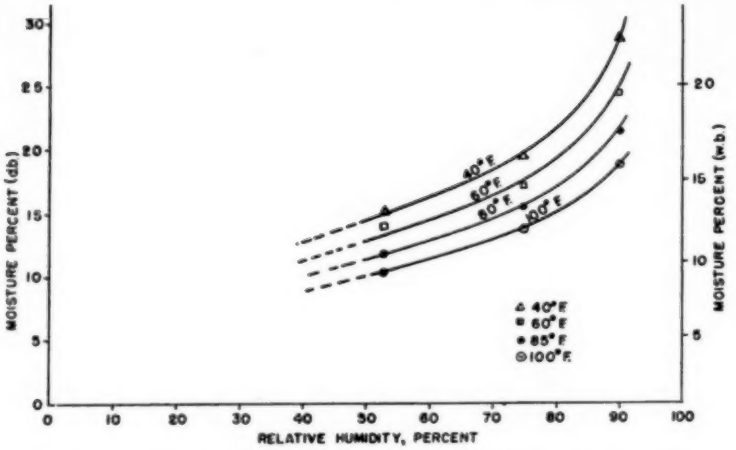


Figure 1.—Equilibrium moisture content of sugar beet seeds (216 x 226).

temperature. Two samples were taken from each of the original samples giving a total of four samples for three-day and seven-day germination tests. The time of exposure began as soon as the seeds were within 10° F. of the oven temperature. At the end of each exposure period the samples were air cooled to room temperature, weighed accurately, and all except 50 grams used for germination tests. The 50-gram sample was used for determining the moisture content. From the information obtained by using a mercury thermometer and thermocouples, it appears that the sample temperature was 1° to 5° F. below the oven temperature.

The three- and seven-day germination tests did not show impairment with oven temperatures of 110° F. for 2 hours and 120° F. for an exposure of 1 hour. For exposures longer than 2 hours, at oven temperatures of 110° and 120° F., the germination was reduced to 47 percent for the three-day and 52 percent for the seven-day germination tests for 6 hours at 110° F. For exposures at 130° and 140° F., the germination was impaired for exposures of 1 hour and longer. Subsequent tests at 115°, 116°, 117°, 118° F. did not reduce the seven-day germination, but did decrease the three-day germination, particularly at 117° and 118° F. for 2 hour exposure and longer.

Air Flow-Static Pressure-Depth Tests

A rectangular bin 8 feet high with a cross-section of 20 inch by 20 inch connected to a variable-speed fan was used for making the air-flow tests. The quantity of air was determined on the discharge side of the bin with a vane anemometer. Air flow readings were taken with sugar beet seeds at a moisture content of 13.5 percent, wet basis, at one-foot intervals from one to seven-foot depth in the bin. Static pressure data were obtained from a pressure tap near the entrance of the air to the bin. The relationship of

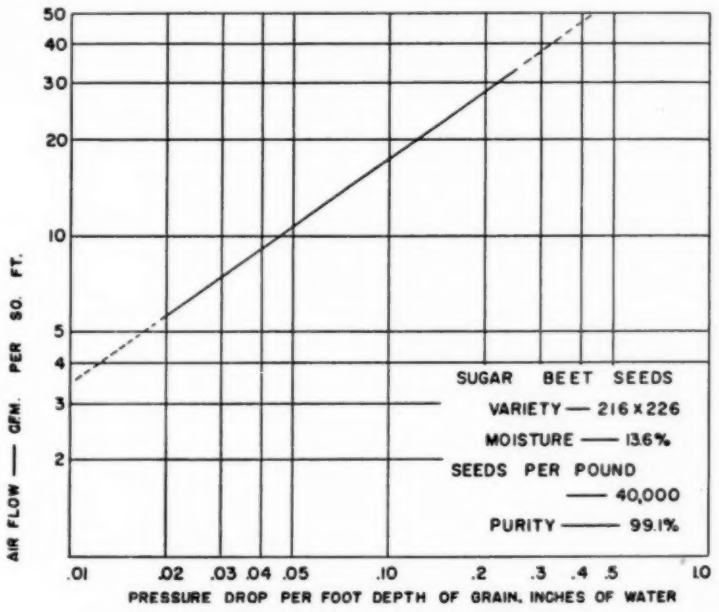


Figure 2.—Resistance of sugar beet seeds to air flow.

air flow in CFM per square foot to the static pressure in inches of water for a one-foot depth of seeds is shown in Figure 2. The data obtained for each one-foot depth were averaged to obtain the line shown in Figure 2. To obtain the static pressure for any other depth, multiply the value for one foot times the depth in feet. Thus, for a three-foot depth of seeds the static pressure for a given air flow would be three times the value obtained from Figure 2. The air-flow data represents the average values obtained when filling and emptying the bin in one-foot increments. Thus, the static pressure values given in the graph are slightly higher than the values which would be received for loose fill only. At a given air flow for sugar beet seeds the static pressure is slightly less than for soybeans and shelled corn. The air flow data are valuable for specifying a fan for moving air through deep layers of sugar beet seeds.

Exposed Drying Rates

The exposed drying rate of a product can be used for determining the effect of temperature and air flow on drying. A thin layer of seeds (the thickness of the diameter of the seed) was placed on the bottom of a three-inch diameter drying chamber. Heated, forced air was supplied by an electric hair dryer. The temperature of the air was changed by varying the resistance of the heater element in the hair dryer. The seed-drying chamber was removed and weighed periodically to determine the amount

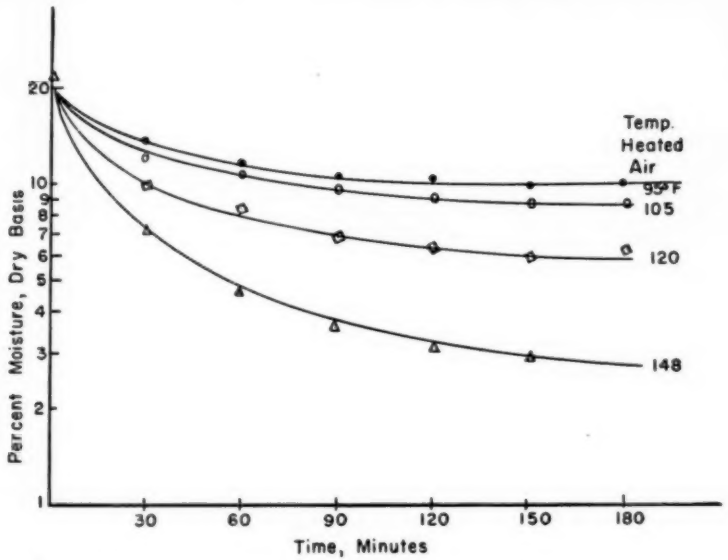


Figure 3.—Exposed drying rate of sugar beet seeds. Original unheated air had: RH-60 percent, average 78° F., air flow—90 feet per minute.

of moisture removed during any given time period. A three-inch diameter-type velocity meter was placed on the top of the chamber to determine air velocity. Both the original and final moisture contents were determined by a gravity oven-dry analysis with the oven set at 212° F. for three days. The exposed drying rates are shown in Figure 3. Air originally at 78° F. and 60 percent relative humidity was heated to 95°, 105°, 120°, and 148° F. Note that a logarithm scale is used for representing the percent of moisture on a dry basis.

Layer Drying

Under practical drying conditions the seeds would be placed in depths greater than a one-seed diameter. Tests were run using a 4-inch depth of beet seeds. The average percent moisture after various periods of drying with heated air is shown in Figure 4, for drying air at 100°, 120°, 143°, 195° F.

Coefficient of Friction

The coefficient of friction of sugar beet seeds at 6.3 and 16.5 percent moisture on several surfaces was obtained by placing the seeds in a small frame, which did not touch the surface being tested, and tilting the table until the seeds slid on the required surface. The tangent of the angle at which the seeds will just slide is the coefficient of friction. The data in Table 1 are the average of five tests.

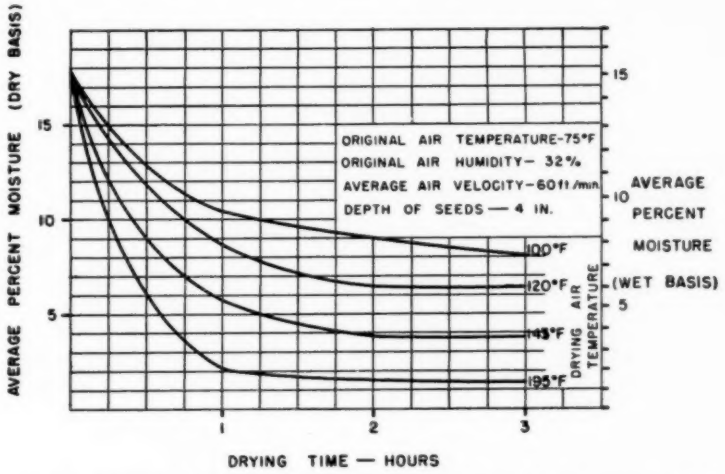


Figure 4.—Drying sugar beet seeds in four-inch layer in laboratory bin.

Table 1.—Coefficient of Friction of Sugar Beet Seeds.

Treatment	Percent moisture 6.3	Wet basis 16.5
Seeds on seeds	0.78	0.81
Seeds on concrete (wood float)	0.81	0.84
Seeds on finished plywood (with grain)	0.69	0.71
Seeds on finished plywood (across grain)	0.70	0.75
Seeds on smooth galvanized iron	0.50	0.55
Seeds on oxidized galvanized iron	0.66	0.71
Seeds on rough cut lumber	0.90	0.97

Conclusions

1. Sugar beet seeds will go to a moisture content above 12 percent (w.b.) as the relative humidity goes above 50 percent and the temperature below 60° F.

2. To remove excess moisture with heated air, 110° F. air is the maximum temperature which should be used if the seeds are exposed to the heat for over 1 hour. The exposure time will depend on the moisture content of the product at a given air flow. To reduce a 4-inch layer of seeds from 14.5 to 11 percent moisture (w.b.) with air at 60 feet per minute required 29 minutes. If accurate controls were used on the heater, and seeds cooled quickly after drying so that the seeds were not heated for more than 1 hour, 120° F. air could be used to dry seeds with 14.5 percent moisture in 23 minutes.

3. The static pressure at a given air flow through the seeds is slightly less than for shelled corn. Similar drying procedures and equipment can be used for sugar beet seeds and shelled corn.

4. The coefficient of friction of sugar beet seeds is considerably higher than for the common cereal grains. More slope is needed to provide movement of seeds in pipes and on various surfaces.

ACKNOWLEDGMENT is made to the Farmers and Manufacturers Beet Sugar Association, Saginaw, Michigan, for financial support; to Perc A. Reeve, Director of Research, and Mark R. Berrett, Agronomist, of the Farmers and Manufacturers Beet Sugar Association for coordination and germination tests, respectively; Maurice Brandt, Department of Agricultural Engineering, Michigan State University, for obtaining the data for one set of the equilibrium moisture values.

Reference

(1) REEVE, PERC A. 1954. Sugar beet seed production. Sugar Beet Journal, 19: 1-2, September.

Experience with Auto-Jet Filters

GEORGE W. COSSAIRT AND LOUIS E. PURMORT[1]

Despite the fact that sugar is the most pure food obtainable, specifications of many industrial users are very exacting. Trace impurities become problems of gross magnitude in their processes. Soluble trace impurities are, principally, a function of white pan purities, which, in turn, are dictated by process economics. Insoluble trace impurities are, of course, related to the effectiveness of thick juice filtration. Two campaigns of operating experience with "Auto-Jet" filters at Santa Ana, demonstrated that filtrate quality and filtration economics were both improved.

As the new filter station has been previously described (1), this paper will be confined to the operational results and techniques of the two campaigns of 1955.

Nearly 800 filtering cycles with the "Auto-Jet" filters have generally confirmed previously reported results (2).

1. Filtrate quality is excellent.
2. Flow rates of 7 to 8 gph/sq. ft. are optimum.
3. Cycle times are normal.
4. Reliable asbestos precoat filter media is easily applied.
5. Filter cakes are quickly and thoroughly sluiced away.
6. Sluicing creates no objectionable distrubances for the beet end.
7. Scale accumulations, which tend to decrease filtering efficiency, are easily removed.

Filtrate Quality

Indices of comparison of filtrate quality were visual sediment discs on all strikes and bacteriological tests on bottlers sugars. Sediment discs are prepared on each strike as a routine control measure. Comparison of 1954 tests with those of 1955 showed a marked improvement. Almost without exception, each of the 1955 strikes were superior to the most exacting specifications.

Bacteriological data provided a more quantitative measure of filtrate quality. Although the sanitary measures throughout the mill were equally good in the 1954 and 1955 campaigns, the data indicated a marked improvement in the number of reject strikes.

	1954	1955
Total strikes tested	264	247
Reject strikes	82	11
Percent reject strikes	31.1	4.5
Average count in all strikes	559	70

Considering that bacteria are in the micron size range, a rather tight filter media was established and maintained on the new filters. On the other hand, permeability was maintained, as verified by the normal filter cycle times. Several factors were responsible for this desirable condition, namely, a proven precoat procedure, minimum abuse of the filter cake, and a favorable personnel situation.

---

[1] Assistant Superintendent, Holly Sugar Corporation, Alvarado, California, and President, United States Filter Company, El Monte, California, respectively.

[2] Numbers in parentheses refer to literature cited.

Personnel

Once the system was settled into its simple routine, it was felt that if a full time operator were needed, a second class station classification would be proper. However, only a few minutes of direct labor were required per shift, thus, the beet end foreman or the extra man performed the necessary details.

With the installation of the "Auto-Jet" filters, interest in the new equipment ran high. As a result, there was an almost unnoticed refresher course in filtration among the plant personnel. These two situations created a very favorable personnel condition. The new system was immediately and closely integrated with the rest of the factory processes.

This unexpected use of top caliber personnel was an intangible, but nevertheless, positive benefit with respect to station performance. As the foremen operated the station incidental to their other duties, there was no dirct labor charge to the filters. A very tangible economic benefit is obvious.

Precoating

Hydraulic application of a reliable filter media of asbestos and filter aid is predicated upon filling the filter shell with a filtered syrup before circulating the precoat slurry. In this way, the precoat material enters the filter and immediately deposits evenly upon the leaves.

For the first thick juice filters, 7½ pounds of grade 50 asbestos was added to about 250 gallons of previously filtered juice. The asbestos was most evenly dispersed by a 3 minute circulated flow through the precoat pump and directly back to the precoat tank. Simultaneously, 50 pounds of filter aid (JM Celite 512 or Dicalite Special Speed Flow) was slurried in about 125 gallons of juice in another mixing tank.

The asbestos slurry was then circulated through the filter at a high flow rate for 3 minutes. The filter aid slurry was then added to the precoat tank and circulated for another 8 to 10 minutes, although excellent clarity was obtained in 5 to 8 minutes. The flow rate was then reduced to normal, and filtration of melter syrups was begun immediately.

For refilters, the procedure was identical except that 10 pounds of asbestos fiber were used.

Filtering

Permeability of the filter cake was maintained by avoiding abuse of the cake. Abuse was minimized by consideration of solids conditioning, optimum flow rates, even density feed syrups, gradual pressure changes, elimination of air, and a continuous and even flow.

To reduce the disturbing influence of air passing through the cake, the filter shells were continuously vented. The melters, pumps, and recirculation systems were modified, where possible, to reduce entrapment of air in the filter feed slurries.

Recirculation of the filtered juice (1, 2) provided an even, continuous flow, despite irregularities in materials entering the high raw melter. Sudden pressure changes were thus avoided.

During the first few weeks, a flow recorder controller aided in evaluation of the filters and confirmation of desirable or optimum flow rates. Filtering times were about 24 hours for the first filters and 80 to 140 hours

for refilters. Flow rates of 7 to 8 gph/sq. ft. were about optimum. Flow rates as high as 12.5 are possible, but cycle times are seriously shortened and filtrate quality is less assured.

With respect to avoiding excessive flow rates, maintenance of full filtering area was a temporary problem. The longer cycle times for refilters permitted time for some organic type scale formation. This scale is common to all types of filters operating on high density melter syrups. For example, this is indicated on plate and frame filters by the loss of softness and pliability of the textile media and by scale accumulations on the plate faces and in the drainage channels.

With the "Auto-Jet" filters, a slow but progressive blinding (due to scaling) was noted on the refilter screens. Fortunately, however, a 1-hour hot caustic recirculation through the filter removed the scale and restored the full filtering area of the screens. Present experience indicates that a caustic cleaning at about 4-week intervals would assure a high level of filter efficiency.

For evaporation economy reasons, high density syrups are desirable. Poiseuille's basic filteration equation warns us, however, of the adverse effects of viscosity upon flow rates. As should be expected, high densities present problems with any filter.

Although a filtering temperature of 98° C. aided in depressing viscosity, there was difficulty in filtering syrups with a Brix much above 65° (hot). At the relatively high-flow rates used (7-8 gph/ sq. ft.) the Brix of the syrups averaged 71.5 (corrected) for the two campaigns.

If much further improvement in syrup density is to be made, it must come primarily from measurement and control of viscosity. Some test work at Alvarado has indicated promise in this respect.

Solids conditioning of the syrups was, of course, continuously reviewed. Filter aid addition to the melters was readjusted, as necessary to maintain the cake porosity, depending upon the beets and the carbon addition. Facilities were provided to add the carbon and filter aid to the high raw melter in a regulated and continuous manner. Filter aid to the refilter supply was also regulated.

Sluicing

A thorough cleaning of the leaves was assured with 3 minutes of sluicing. Only 1,000 to 1,100 gallons of thin juice were used as the sluice fluid. The sluiced cake was drained to the carbonation Oliver filter mud tank. This system caused no dilution to the beet end and no appreciable disturbance to the Oliver filters.

Following the campaign, the leaves were all examined. The first filter screens were discolored but not blinded. The refilters had a small percentage of scale blinded area. A caustic treatment, a five-minute, dilute-muriatic acid rinse, and a final soda neutralizing rinse removed all organic and carbonate scale. The screens were then as bright and clean as when initially installed.

References

(1) COSSAIRT, G. W. 1955. Self-sluicing unit points way to pushbutton filtration. Food Eng. 27:59-61.

(2) COSSAIRT, G. W. 1953. Observations on leaf type filters, Calif. Field Crops Tech. Conf., Opr. Dept. Session. 69-118.

Breeding of Sugar Beets With Reference to Sodium, Sucrose, and Raffinose Content

R. E. Finkner and H. M. Bauserman[1]

In recent years, new techniques and laboratory equipment suitable for mass operation in sugar beet chemical analysis have been developed. For example; flame spectrophotometry for the determination of various cations and the paper chromatographic technique for the determination of raffinose and other melassigenic substances.

The literature before 1930 contains numerous references to non-sugars but very little data applied directly to a breeding program. Such references were briefly reviewed by Doxtator and Calton (2). Later references using chemical analysis other than sucrose in the breeding phases of the research work have been summarized by Wood (5).

Many investigators (1, 2, 3, 4, 5) have applied selection pressure for low sodium content of individual roots. All have shown that the sodium content was significantly correlated with sucrose but in a negative relationship. All have shown by progeny tests that significant reductions in sodium could be accomplished by mass selection. In the majority of such tests, significantly higher sucrose percentages have been experienced. However, in very few investigations has a low sodium selection produced progenies which significantly out yielded their original parents in pounds sucrose per acre.

Wood (5) reported on progeny tests of roots selected for high and low raffinose content. He concluded that the raffinose content of beets could be significantly reduced by mass selection and that this character may be rather simply inherited.

The objectives of the present investigations were to separate the original population into groups with distinctly different quantities of tested chemical constituents and to determine the effectiveness of using low sodium as a criterion in selecting for higher sucrose content.

Methods and Materials

Approximately 1,200 roots of an elite stock of American No. 1 were selected during the fall of 1953. These roots were selected phenotypically for size, shape, good crowns, etc. Each root was weighed, sampled, and analyzed for sucrose, raffinose, sodium, galactinol, and inositol.

Table 1 shows the correlation coefficients of the various characteristics studied in the original selected population. Sucrose and sodium were reported as percent of beet, while raffinose, galactinol, and inositol were percent on dry substance; the weight was reported in pounds.

These roots were separated into three main groups of approximately 400 roots each. The three groups represented a random mixture of the original variety and did not differ greatly from each other as every beet had an equal chance of being selected for any one of the three groups.

---

[1] Chief Plant Breeder and Head Research Chemist, respectively, American Crystal Sugar Company, Rocky Ford, Colorado.

[2] Numbers in parentheses refer to literature cited.

Table 1.—Correlation Coefficients of 1046 Roots Selected from an Elite Stock of American No. 1.

	Raffinose	Sodium	Galactinol	Inositol	Weight
Sucrose	—.1412	—.7115	—.3483	—.1612	—.2322
Raffinose	.....	.0964	.0663	.1412	—.0096
Sodium	.....	.....	.2473	.1122	.1909
Galactinol	.....	.....	.....	—.0021	.0068
Inositol	.....	.....	.....	.....	.0845

r = .062 for significance at the 5 percent level.
r = .081 for significance at the 1 percent level.

Each of these main groups were again divided into two smaller subgroups, altogether, making a total of six small groups. A seventh group was added by selecting approximately every 35th beet regardless of its physical and/or chemical characteristics as a check selection with which the other groups could be compared. This group was labeled 54-407.

The first group was subdivided by selecting paired roots with the same sucrose content, but one (54-408) having a high sodium content and the other (54-409) having a low sodium content. The second group was subdivided by selecting for high and low raffinose content. These were given pedigree numbers of 54-410 and 54-411 respectively.

In the last group the beets were divided into two groups, odd and even numbers. Thirty-one roots were selected within the even numbers on a combined basis of both sucrose and sodium percent. The coefficient used for this selection was sucrose percentage times (one minus sodium content). Within the odd numbers, thirty beets were selected on the basis of high sugar only. These were given the numbers of 54-412 and 54-413 respectively.

All other characteristics were recorded but only the ones mentioned above were used in differentiating this population into the various subgroups.

The number of roots selected for each group, the general means, and standard errors of means of each character studied for each group, and for the entire population, are shown in Table 2.

Table 2.—Means, Standard Errors, and Number of Roots of the Entire Population, and of the Seven Selected Groups for Various Chemical Characteristics.

Pedigree Number	Criteria for Selection	No. Roots Selected	Weight in Pounds	Percent Sucrose	Percent Sodium	Percent Raffinose
54-407	Check	32	2.63±.15	13.18±.28	.1129±.0095	.434±.0249
54-408	High Sucrose—High Na.	32	2.28±.12	15.68±.11	.0916±.0055	.426±.0332
54-409	High Sucrose—Low Na.	32	2.09±.10	15.67±.11	.0341±.0017	.458±.0390
54-410	High Raffinose	38	2.58±.18	12.74±.40	.1043±.0087	.841±.0211
54-411	Low Raffinose	39	2.53±.15	13.71±.26	.0898±.0074	.158±.0062
54-412	A Coef. of Na. and Sucrose	31	2.07±.09	15.82±.12	.0495±.0035	.387±.0272
54-413	High Sucrose	30	2.24±.12	15.92±.13	.0606±.0048	.478±.0414
Entire Population		1046	2.53±.03	13.69±.08	.0972±.0015	.457±.0061

Roots of each of the above seven groups were space isolated in the spring of 1954 and produced seed that fall. Seed from these seven groups plus seed of the original parental stock were planted in an 8x8 Latin Square test at Rocky Ford, Colorado, on April 25, 1955. Plots were 4-rows wide and 35-feet long. The two center rows were harvested and three 10-beet samples were taken at random from the harvested roots.

Three weeks later border rows of five of the selections were harvested and two 10-beet samples were taken for chemical analysis. The objective of the second harvest was to determine whether or not the raffinose content increased while the beets were still growing in the late fall.

Results and Discussion

The first harvest results of the 1955 replicated progeny test of the various chemical selected strains are shown in Table 3, along with the original parent. All seven of the chemical selections originated from the same parental variety and only one mass selection was made to differentiate these strains into their respective sub-populations.

Highly significant differences were obtained among the various groups for every attribute listed in Table 3. This was as expected as selection pressures were applied in opposite directions for some of the chemical characteristics such as raffinose.

Table 3.—Stand, Yield, and Chemical Results of the Parent and Seven Selected Chemical Strains.

Pedigree Number	Criteria for Selection	Pounds Sucrose Per Acre	Tons Per Acre	Percent Sucrose	Percent Na.	Percent Raffinose	Number of Beets Per 100 Feet
54-407	Check	8251	26.93	15.32	.0572	.214	84.1
54-408	High Sucrose—High Na.	7868	24.71	16.01	.0554	.248	101.2
54-409	High Sucrose—Low Na.	8137	25.32	16.05	.0314	.273	96.1
54-410	High Raffinose	6838	22.06	15.48	.0468	.336	93.7
54-411	Low Raffinose	7714	24.64	15.66	.0421	.164	94.5
54-412	A Coef. of Na. and Sucrose	8451	27.15	15.64	.0401	.217	87.8
54-413	High Sucrose	7951	24.66	16.05	.0405	.237	94.1
Parent	American No. 1	7225	23.62	15.24	.0534	.250	101.0
General Mean		7804	24.89	15.68	.0459	.242	94.1
LSD 5% Pt.		638	2.07	.46	.0084	.048	7.8
LSD 1% Pt.		853	2.77	.62	.0113	.064	10.4
C. V.		2.87%	2.92%	1.03%	6.52%	6.90%	2.90%

Certain paired comparisons of the various strains should be considered because most of the groups were selected with this in mind. The comparison of the parental variety with the check (54-407) shows a highly significant increase for tons and pounds sucrose per acre in favor of 54-407. Strain 54-407 represents a random mixture of the selected roots from the parental variety. If fiducial limits were calculated for the means in Table 2 for selection 54-407 and the entire population, they would overlap. This indicates that selection 54-407 was a satisfactory sample of the entire population of 1046 root selected from the parental American No. 1 variety.

The results in Table 3 show that the progeny (54-407), with one phenotypic selection for large well-shaped roots, bred true and the root

yield was significantly increased over the parental American No. 1 variety. There were no significant differences between these strains for any of the chemical characters studied. Although significant differences were detected in stand, it did not seem to affect either the yield of tons and/or sucrose percentage. Evidently the plants were sufficiently spaced to have adequate competition.

Because selection 54-407 was part of the same population of selected beets which made up the other strains in this test and because it was significantly higher than the parental strain for tons, and pounds sucrose per acre, it should be used as the check in the various comparisons to be discussed.

One of the objectives of the experiment was to select for high and low raffinose content and then test the progenies to see if they bred true. In Table 2, the means for raffinose content for selected groups 54-410 and 54-411 were significantly different from each other. If fiducial limits were calculated at the one percent level, the raffinose means for these two groups would not overlap. The selected roots of 54-410 also contained highly significantly more raffinose than the check 54-407, while the roots of 54-411 were significantly below the check.

The progeny results in Table 3 show these two selections differ significantly at the one percent level for percent raffinose. The check selection with a mean of .214 was significantly higher at the five percent level than 54-411 with a mean of .164. However, the check was significantly lower at the one percent level than the high raffinose selection 54-412. The first cycle of selection indicated that these chemicals were hereditable and greater advances could be made in breeding for a higher raffinose variety than for a lower raffinose strain.

The correlation coefficients in Table 1 show a negative relation for raffinose with sucrose and weight, however, the latter was not significant and the value was so low that weight and raffinose could be considered independent of each other. Sodium had a low positive correlation which was expected because of its negative relationship with sucrose. The means of the selected roots in Table 2 verified these correlations as the low raffinose selection (54-411) was lower in sodium and higher in sucrose percentage than 54-410; weight was approximately the same. However, none of these means, including the check, were significantly different at the five percent point.

The progeny test of Table 3 also confirms the above correlation with the high raffinose selection, 54-410 being slightly higher in sodium and lower in sucrose and weight than the low raffinose selection. The progeny of 54-411 produced significantly more tons and pounds sucrose per acre than 54-410. This might indicate that the negative correlation between raffinose and weight was stronger than the value obtained in Table 2. It also may have been due to environmental conditions. Another possibility was that either extremely high or low raffinose content was detrimental, as the check yielded significantly higher tons than these extreme raffinose selections. The check was significantly higher than the low raffinose selection at the five percent level and the high raffinose selection at the one percent level. It also was significantly higher in sodium percentage and slightly lower in sucrose.

The border rows of five of the selections were harvested three weeks later and two 10-beet samples were taken for chemical analysis. These are shown in Table 4.

Table 4.—Chemical Results of the Late Harvest of the Five Strains.[1]

Pedigree Number	Criteria for Selection	Percent Suc.	Percent Na.	Percent Raff.	Increase of Raffinose in 3 Weeks
54-408	High Sucrose—High Na.	17.08	.0455	.300	21.0%
54-409	High Sucrose—Low Na.	17.06	.0296	.353	29.3%
54-412	A Coef. of Na. and Sucrose	16.20	.0419	.300	21.0%
54-413	High Sucrose	17.16	.0372	.314	32.5%
Parent	American No. 1	16.29	.0510	.318	27.2%
General Mean		16.76	.0411	.317	29.6%
LSD 5% Pt.		NS	.0139	.029	.....
LSD 1% Pt.		NS	.0188	.039	.....
C. V.		2.01%	11.71%	3.12%	.....

[1] Analyzed as a randomized block design with eight replications and five varieties.

The raffinose data was analyzed by assuming a split plot design with dates as the sub-plots and varieties as the main plots. Highly significant differences were found among the varieties and the F value for dates was 76.4. In order to be significant at the one percent level the F value only needed to be 7.4. The date X variety interaction was very small and non-significant.

What caused this increase in raffinose we do not know, but we assume that the colder weather had some effect. The mean minimum temperature for two weeks before the first harvest beginning October 1 was 40.9 degrees, while the minimum mean temperature between the two harvests was 30.3 degrees Fahrenheit. There were only three days which were 32 degrees or below before the first harvest, and eleven days which were 32 degrees or below between the two harvests. The dates of the two harvests were October 14 and November 4.

Additional experiments will be necessary to determine the effect of environment on the accumulation of raffinose.

The remainder of the test was designed to determine the effectiveness of using sodium in the breeding program as a criterion in selecting for higher sucrose content.

The fact that sodium and sucrose were negatively correlated to a very high degree, as shown in Table 1, suggests the possibility of using both of these characteristics to select for high sucrose content. The hypothesis is, that the mechanism of the beet was such that low sodium, per se, directly or indirectly, tended to bring about a higher concentration of sucrose or visa versa.

The comparison of strains 54-408 and 54-409 were selected with the same sucrose content but with a high and low sodium content. The means in Table 2 indicate the groups were selected in that manner as the sodium content of these groups was significantly different, but all other characteristics were the same.

The results of the progeny test are given in Table 3. Two main points should be considered; first, the sucrose content of these two groups were within .04 percent of each other in Table 3, which was the average of 24 samples, and with .02 percent of each other in Table 4, which was the mean of 16 samples. The objective of holding sucrose constant during the selecting of mother roots was fully realized in the progeny test. The second point to consider was the sodium content. The results of the progeny test in Table 3 show the mean sodium percentage for 54-408 as .0554 and for 54-409 as .0314. These means were significantly different at the one percent level. Similar results are shown in Table 4. No other significant differences were detected for these two groups. In comparing these two selections (54-408 and 54-409) with the check we find differences among the following characteristics: (A) The sucrose content of both selections was significantly higher than the check at the one percent level, (B) selection 54-408 was significantly lower than the check in tons per acre, (C) selection 54-409 was highly significantly lower in sodium percent than either the check or selection 54-408, ad (D) selection 54-409 was significantly higher in raffinose than the check.

The fact that the low sodium selection did not increase the sucrose content indicates that these two chemical mechanisms were not as directly related as first thought, and that sugar beet varieties could be bred with a high sodium and a high sucrose content. However, a strict interpretation of the sucrose-sodium correlations in Table 1 shows only 50 percent of the factors which affect low sodium have an effect on high sucrose. Perhaps the environmental factors have great effect on these two characters. Again additional investigations are needed to study the inheritance of sucrose and sodium. Such experiments also should be designed to study the environmental effect and the genetic-environmental interactions.

The last comparison was designed to test two methods of selection for high sucrose content. Group 54-412 was selected on the basis of a sodium-sucrose coefficient while group 54-413 was selected only on the basis of high sucrose. If low sodium was a factor in selecting for high sucrose, then the progeny of group 54-412 should contain a higher percent of sucrose than group 54-413.

Again if fiducial limits at the five percent point were calculated for the means of groups 54-412 and 54-413 in Table 2, all would overlap. This would indicate that the selected roots of these two groups were part of the same population for all characteristics shown in Table 2.

The results of the progenies from these two groups are shown in Table 3. The sodium content of these two groups were approximately equal. Therefore, selection for high sucrose was just as effective for lowering the sodium content as selection on a sucrose-sodium coefficient. The sucrose means were 15.64 for group 54-412 and 16.05 for group 54-413. The difference between these means was .41 while .46 was needed for significance. Although we can not say that these populations are different at the five percent point, it is certainly questionable, whether they are estimates of the same population. The results shown in Table 4 also favor selection 54-413 over 54-412 for higher sucrose content, but again it was not statistic-

ally significant. However, over 40 samples were taken from these strains and the trend of 54-413 was slightly higher in sucrose. A comparison of the check 54-407 with these two selections shows 54-413 to be significantly higher in sucrose content than the check, while selection 54-412 was not statistically different from the check. If this comparison were used, it would indicate that selecting for high sucrose content was more effective than using a sodium-sucrose coefficient in obtaining progenies with a higher sucrose content.

The weight factor between these groups was in favor of group 54-412. The mean ton yield of group 54-412 was 27.15 while group 54-413 yielded only 24.66. This difference was significant at the five percent level. Very few investigators in the past have reported an increase in weight in low sodium selections, and the correlations in Table 1 show that such a result is unlikely. However, the data in this test in Table 3 show higher yield in tons for both of the low sodium selections (54-409 and 54-412) when compared to their respective counterpart (54-408 and 54-409). When groups 54-412 and 54-413 were compared for gross sucrose per acre, no significant differences could be detected. A "t" test was applied to find the level of significance for these two groups in pounds sucrose per acre. A "t" value of .756 with 14 degrees of freedom was calculated. The probability that these means were different was less than 40 percent.

Three points should be considered from the results of the sodium test. First, the sodium content was heritable and a low sodium variety of sugar beet can be bred if such was desirable from the processing view point. Second, it was possible to breed for various levels of sodium without effecting the sucrose content. Third, the selection based on sucrose only produced progenies which were slightly higher, although not significantly, in sucrose than the progenies of a sucrose-sodium selection.

In view of the above facts it seems at the present time that sodium was actually of little value in the breeding program and that a high sucrose selection could be accomplished without selecting for low sodium. Also, the fact that sucrose percentage was measured with more precision than sodium, as shown by the C. V. values, emphasized the need of sucrose reading without combining it with a sodium reading which has a large variance.

Summary

Approximately 1,200 roots of an elite stock of American No. 1 were selected in the field in 1953. These roots were subdivided into seven groups. Six of the groups were selected for distinctly different chemical contents and the seventh group represented a random mixture of the 1,200 selected roots.

From the data submitted in this report the following conclusions were drawn.

1. It was possible to select roots with high and low raffinose content and these bred true in progeny tests.

2. The raffinose content of selections in the field was found to have increased approximately 30 percent in three weeks. Lower minimum mean temperatures were suggested as one possibility for causing this increase.

3. Paired roots were selected with the same sucrose content but with one having a high sodium content and the other having a low sodium content. Progenies from these selections bed true, i.e., both had equal sucrose content but significantly different sodium percentages. This indicated that sodium, per se, had little effect on the sucrose content and that the negative correlation of sucrose and sodium could be easily broken.

4. Progenies selected only on the basis of their sucrose content had a higher sucrose content than progenies selected on the basis of a sucrose-sodium coefficient.

5. In view of the data at the present time it seems that sodium actually is of little value in the breeding program, unless a low sodium variety is extremely important from the processing viewpoint.

References

(1) DAHLBERG, H. W. 1950. Chemical methods for breeding sugar beets. Proc. Amer. Soc. Sugar Beet Tech. pp. 137-138.

(2) DOXTATOR, C. W. and CALTON, F. R. 1950. Sodium and potassium content of sugar beet varieties in some western beet growing areas. Proc. Amer. Soc. Sugar Beet Tech. pp. 144-151.

(3) DOXTATOR, C. W. and BAUSERMAN, H. M. 1952. Parent-progeny tests for sodium and potassium content. Proc. Amer. Soc. Sugar Beet Tech. pp. 319-321.

(4) BROWN, R. J. and WOOD, R. R. 1952. Improvement of processing quality of sugar beets by breeding methods. Proc. Amer. Soc. Sugar Beet Tech. pp. 314-318.

(5) WOOD, R. R. 1954. Breeding for improvement of processing characteristics of sugar beet varieties. Proc. Amer. Soc. Sugar Beet Tech. VIII (2):125-133.

Resistance of Inbred Varieties of Sugar Beets to Aphanomyces, Rhizoctonia, and Fusarium Root Rots[1]

M. M. Afanasiev[2]

Several hundred varieties of sugar beets were tested for their resistance to Aphanomyces, Rhizoctonia, and Fusarium root rots during 1950 to 1955. Among these varieties were inbred lines and open pollinated commercial varieties. Although some of the commercial varieties showed a high degree of resistance to some of these root rots, it was decided not to test them further because they do not genetically represent a uniform population. Because of this, it is impossible to duplicate the same results with their progenies. Therefore, the main emphasis in this work was placed on testing the reaction of inbred varieties to root rots.

The inbred varieties of sugar beets were supplied by the Sugar Beet Breeders Forum. The following method was adopted for testing resistance of beet varieties to Aphanomyces, Rhizoctonia, and Fusarium root rots. Each variety of beets was tested for each disease in four seven-inch pots filled with Huntley soil. Prior to each planting, pots filled with soil and saucers were steam sterilized for four hours at 15 pounds pressure. Usually, 20 whole beet seeds were planted in each pot. Seeds were disinfected with New Improved Ceresan about one month prior to planting. Each pot of soil can be considered as a replication. Soil was inoculated immediately after the seeds were planted. The inoculum was composed of several cultures of each of the following pathogenic organisms whose pathogenicity was previously proven: *Aphanomyces cochlioides* Drechs.; *Pellicularia filamentosa* (Pat.) Rogers, (*Rhizoctonia solani*); and *Fusarium oxysporum* f. *betae* (D. Stewart) Snyder and Hansen. The beets were harvested about six weeks after emergence. Readings of healthy and diseased beets were taken during the growing period and at harvest.

Only those varieties which showed a uniformly high degree of resistance in all four replications were saved for further testing, the remaining varieties were discarded.

Five young beets of the resistant varieties were transplanted in individual pots and were grown until their roots were about one to two inches in diameter. They were then placed in a cold room for vernalization for about 10 weeks. Following this treatment the roots were sent to either D. F. Peterson of the Holly Sugar Corporation or H. E. Brewbaker of the Great Western Sugar Company for seed production under inbred conditions in the field. The seed produced from these beets by the two cooperators will be returned to Bozeman and again submitted to a pathogenicity test for a specific disease for which the beets were selected. If the progenies of the beets retain a high degree of resistance, they will be considered resistant.

---

[1] Contribution from Montana State College, Agricultural Experiment Station, Bozeman, Montana. Paper No. 372 Journal Series.

[2] Plant Pathologist, Department of Botany and Bacteriology, Montana Agricultural Experiment Station, Bozeman, Montana.

The seed stock will be returned to the agency which furnished the original material.

It has been observed that sometimes the complete test, in which a large number of beet varieties was tested, showed a uniform low percent of disease for a certain root rot. It is believed that some environmental conditions must be responsible for this situation. The same cultures of fungi used prior to this test, or afterwards, usually give a good pathogenicity test. The only way to clarify this point is to repeat the test.

To make sure that cultures retain their high level of virulence, their pathogenicity was regularly checked in separate tests. Those cultures which became low in pathogenicity were usually discarded and new isolations were made from infected seedlings.

The results of testing sugar beet varieties for resistance to Aphanomyces root rot are rather disappointing up to the present time. From several hundred varieties of beets tested, not a single one showed a sufficiently high degree of resistance. A number of varieties submitted by other investigators as being somewhat resistant to this disease completely failed to show resistance in our tests. Very often in the test in which two to three dozen beet varieties were tested, which involved 2 to 3 thousand young beets, only about a dozen beets remained free of disease. It is not known whether these beets were resistant or just escaped being infected. These surviving beets are being used for production of seed. The progeny will again be used in pathogenicity studies.

Better results were obtained in testing beet varieties for their resistance to Rhizoctonia and Fusarium root rots than to Aphanomyces. Among several hundred varieties of beets tested for these diseases, 19 varieties showed a high degree of resistance to Rhizoctonia root rot and 22 varieties to Fusarium root rot. Four varieties showed resistance to both Rhizoctonia and Fusarium root rots. Progenies of most of these beets will be tested for resistance to these diseases during the winter of 1955-56.

The difficulty involved in finding resistance to Aphanomyces root rot is probably due to the nature of the fungus. Sexual reproduction occurs very readily, so there is always the possibility of the formation of new physiologic strains which may differ in pathogenicity. The presence of physiologic strains complicates this type of work.

It is known that sexual reproduction occurs in *Pellicularia filamentosa*, therefore it is possible that new physiologic strains may also arise in this fungus.

Fusarium oxysporum f. *betae*, which is responsible for Fusarium disease of beets, belongs to the Imperfect Fungi. It does not have sexual reproduction. Due to lack of many physiological strains, it will probably be easier to find resistance in beets to this disease than to Aphanomyces or Rhizoctonia root rots. However, the possibility of the occurrence of mutants in Fusaria, which may also have different pathogenic properties, should not be overlooked.

Some Effects of Excess Water Application on Utilization of Applied Nitrogen by Sugar Beets[1]

J. S. Robins, C. E. Nelson and C. E. Domingo[2]

Over-irrigation is a major problem in many irrigated areas. On the new lands in the Columbia Basin Project, most of the soils have relatively high water holding capacities and infiltration rates. Applying irrigation water too frequently and for excessive durations on these soils has made this problem particularly acute.

In addition to contributing to drainage problems in the area, over-irrigation may, by leaching, materially affect the response of crops to fertilizers. This is especially true of nitrogen, which is the nutrient most commonly deficient in these soils.

The effects of over-irrigation in the area have been most noticeable in sugar beet production because of the long irrigation season, the high nitrogen requirement of the crop, and the low nitrogen content of the soils in the area. In many fields, this crop has developed severe nitrogen deficiency symptoms in midseason even where nitrogen applications were relatively high.

Most of the reported irrigation and nitrogen fertilization work on sugar beets has been designed to determine either the effects of depleting the soil moisture to predetermined levels or the effects of nitrogen rates and placement methods on yield, sugar content and purity. Some attention also has been given to certain interrelationships between soil moisture and nitrogen fertilization.

Little evidence exists, however, relative to nitrogen utilization under various conditions of irrigation practice. This is especially true of practices involving application of excess irrigation water[3].

The study reported here was designed to determine the effects of application of excess irrigation water on utilization of nitrogen and the resulting effects upon sugar beet yield, sugar content, and nitrogen status.

Certain of the yield and water application efficiency information has been previously reported (2, 3)[4]. This paper presents two season's data pertaining primarily to nitrogen status and sugar content of sugar beets as affected by excess water application and by nitrogen rate and placement method.

1953 Experiments

Experimental Procedure

Six experiments were conducted in 1953 in the Columbia Basin Project in Washington. At each site, three irrigation treatments were established as follows:

---

[1] Contribution from the Western Soil and Water Management Section, ARS, USDA, in cooperation with the Washington Agricultural Experiment Stations, the Utah-Idaho Sugar Company, and the Amalgamated Sugar Company. Material financial assistance from the Utah-Idaho Sugar Company and the Amalgamated Sugar Company is gratefully acknowledged.

[2] Soil Scientist, Agronomist, and Soil Scientist respectively, Western Soil and Water Management Section, ARS, Irrigation Experiment Station, Prosser, Washington.

[3] "Excess irrigation water" as used in this paper refers to irrigation water added in excess of that which the soil will retain.

[4] Numbers in parentheses refer to literature cited.

Treatment Number	Relative Quantity of Water Added per Irrigation
1	X
2	2X
3	3X

The magnitude of the quantity "X" was determined from pan evaporation measurements (1) in conjunction with plant size and cover. Irrigation frequently was determined from the quantity "X" and the moisture retention characteristics of the profiles. All three treatments at a given site were irrigated simultaneously at frequencies of 14 to 28 days.

Nitrogen rate sub plots were established in each main irrigation plot by sidedressing about thinning time. These rates were 60, 120, 180, and 360 pounds per acre of N at four of the six sites. At the remaining two sites, pre-planting applications of about 120 and 155 pounds per acre of N had been made so the final rates will not conform to the other sites. All nitrogen was applied as NH_4NO_3.

Fifteen to 20 recently-matured petioles were obtained periodically from each plot for nitrogen status evaluations. Two rows 25 to 30 feet in length were harvested for yield and sugar content evaluations.

Results and Discussion

Effects on yield.

Yield and irrigation information for 1953 have been previously reported (2). Deep percolation losses for the 2X treatment varied from 7 to 20 inches and for the 3X treatment from 18 to 39 inches at the six locations. Even with deep percolation losses of these magnitudes, no significant yield depressions were apparent. As will be discussed later, failure to remove sufficient nitrogen to depress yields is attributed to the fact, that little excess irrigation water was applied early in the growing season.

Effects on sugar content

Results of sugar content determinations at harvest for certain of the 1953 locations are presented in Table 1. No measurable effect of irrigation treatment was noted and in general only the highest nitrogen rate resulted in appreciable depression of the sucrose percentage.

Table 1.—Sugar Content at Harvest as Influenced by the Irrigation and Nitrogen Rate Treatments for Certain of the 1953 Locations.

	Sucrose Percentage							L.S.D.
	Irrigation			N Rate (lbs./acre)				(5%)
Location	X	2X	3X	60	120	180	360	N Rate
1	16.3	16.7	16.5	17.4	16.6	16.4	15.6	0.61
3	18.4	18.5	18.2	18.7	18.7	18.7	17.4	0.65
5	18.7	18.5	18.8	19.3	19.0	18.6	18.2	0.62
				N Rate (lbs./acre)				
				155	180	240	360	
4	18.2	17.8	17.9	18.1	18.2	18.2	17.2	0.71

Effects on plant nitrogen level

Results of total- and nitrate-nitrogen determinations on petiole samples are depicted in Figures 1 and 2 for location 1. All nitrogen levels, except the 360 pound rate, were either below or approaching the critical level of Ulrich (4) by September 3, which was about seven weeks before harvesting. This explains the relatively small sugar content differences obtained.

Of particular interest are irrigation-sampling date interactions obtained in these measurements. The irrigation treatments receiving excess water had the highest nitrogen levels in the beet petioles at the first sampling. Differences were greatest at the two highest nitrogen rates indicating that the excess water applied prior to the first sampling date increased the availability of the shallow sidedressed nitrogen. Lower nitrogen levels noted on treatments receiving no excess water were probably due to one or both of two factors: (A) Excessive fertilizer concentration in the area of placement restricting root growth until the nitrogen was moved by the irrigation water, or (B) Dry soil in the surface layer restricting root activity and nitrate movement.

1954 Experiments

Experimental Procedure

In 1954, three experiments were conducted; two near Moses Lake, Washington, in the Columbia Basin Project and one at Toppenish, Washington, in the Yakima Valley. At Toppenish, the irrigation treatments were the same as those used in 1953. At the two Moses Lake locations, the following irrigation treatments were established.

Location 1—Moses Lake

Treatment Number	Irrigation Frequency	Relative Quantity of Water Added per Unit Time
1	2 weeks	X
2	4 weeks	X
3	2 weeks	2X
4	4 weeks	2X
5	2 weeks	3X

Location 2—Moses Lake

Treatment Number	Irrigation Frequency		Relative Quantity of Water Added per Unit Time	
	Before 7/15	After 7/15	Before 7/15	After 7/15
1	4 weeks	4 weeks	X	X
2	2 weeks	4 weeks	2X	X
3	2 weeks	4 weeks	4X	X
4	4 weeks	2 weeks	X	2X
5	4 weeks	2 weeks	X	4X

The quantity "X" was again determined from pan evaporation measurements (1), plant size, and cover. Since fixed irrigation frequencies were used on different treatments, the quantities of water added at each irrigation were determined from the irrigation frequency and the magnitude of

"X" during the interval in question. The frequencies used were determined from soil moisture retention characteristics of the profiles and peak consumptive use information previously determined.

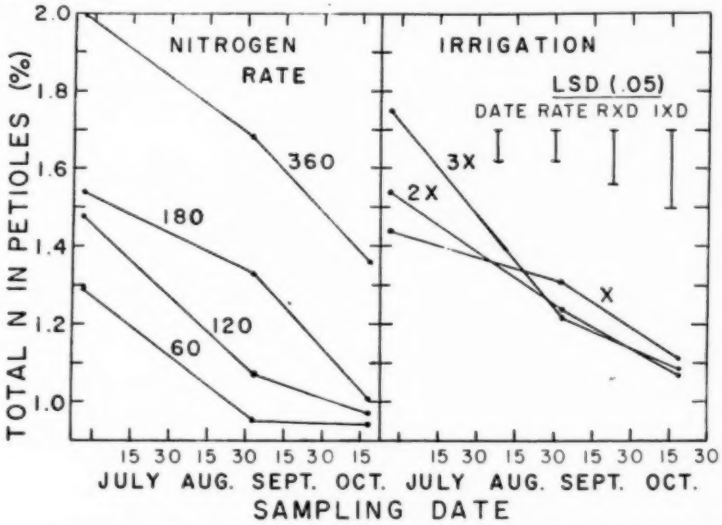


Figure 1.—Effects of nitrogen and irrigation on total-nitrogen level of beet petioles at location 1, 1953.

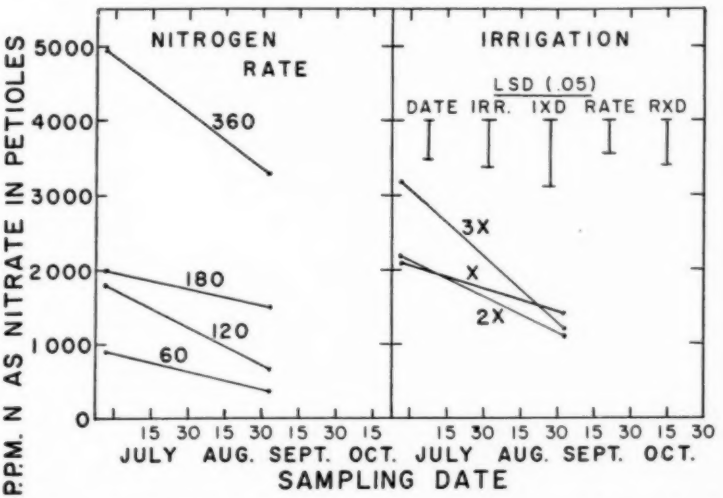


Figure 2.—Effects of nitrogen and irrigation on nitrate-nitrogen levels of beet petioles at location 1, 1953.

Nitrogen was applied at three rates by each of four methods of placement within each irrigation plot. The rates, in addition to the O N plots, were 120, 240, and 360 pounds of N per acre; the placement methods were plowed-down, disced-in, shallow sidedressed, and deep sidedressed. All nitrogen was applied as NH_4NO_3.

Fifteen to 20 young mature petioles were again obtained periodically from each plot for nitrogen status evaluations. Two rows, 20 or 25 feet in length, were harvested for yield and sugar content determinations.

Results and Discussion

Effects on yield

Yield and irrigation information for the 1954 experiments has been reported previously (3). Yields were again unaffected by excess irrigation water at the Toppenish location, which received the same three irrigation treatments as were used in 1953.

The irrigation phases of the remaining two locations were designed to study the effects of higher rates of deep percolation and to compare early-season and late-season applications of excess water. Yield data for these two locations (1954) are presented in Figure 3.

Marked reductions in yield resulted from excess water applications at both locations. Failure to obtain such depressions in yield by late excess applications at location 2 indicates that only the early season applications are important in reducing yield by leaching of nitrogen. This explains the failure to obtain yield depressions in 1953 and at the Toppenish location in 1954, since no early excess water applications were made in these cases.

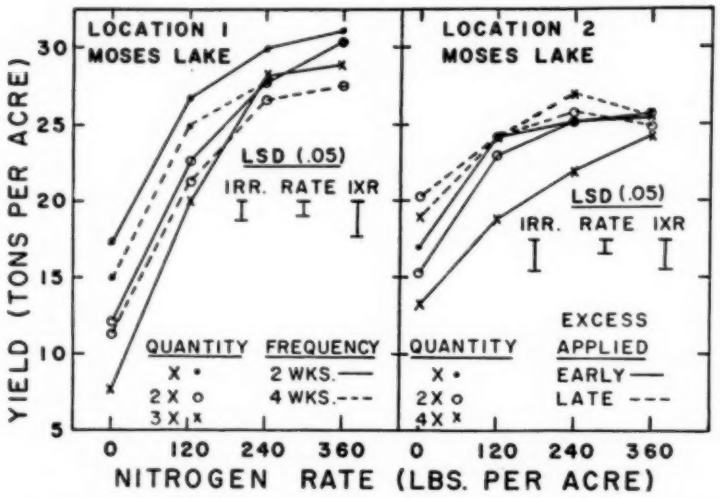


Figure 3.—Effects of irrigation and nitrogen rate on yield of beets at two locations, 1954.

Yield reductions resulting from excess water were considerably greater at low rates than at high rates of nitrogen. This behavior could be expected since a large excess of nitrogen was available at the higher rates. From this supply, the crop could accumulate a high concentration before leaching of nitrogen from the root zone limited the supply. Also, a considerable portion of the applied nitrogen probably was still in the ammoniacal form when the early water applications were made.

An apparent moisture response was noted between the two- and four-week frequencies at location 1. This indicates a possible confounding in the location 2 data, since different frequencies were used at this location also. This probably accounts for the tendency for higher yields on the treatments receiving late excess applications at location 2.

Placement effects are shown in Table 2. No difference in yield was obtained at location 2 as a result of placement. At location 1 and at Toppenish, however, disced-in applications were inferior to the other three methods of application and at Toppenish, the plowed-down application was superior to the sidedressed treatments. These differences in behavior are

Table 2.—Effects of Nitrogen Placement on Yield of Beets for Thee Locations, 1954.

Placement Method	Yield (Tons per Acre)[1]		
	Loc. 1—Moses Lake	Loc. 2—Moses Lake	Toppenish
Plowed-down	27.4	24.3	29.1
Disced-in	24.7	24.3	26.3
Sidedressed, shallow	27.5	24.2	27.9
Sidedressed, deep	28.0	24.7	27.9
L.S.D. (5%)	1.2	N.S.	0.9

[1] Mean yield for 120, 240, and 360 lbs. per acres N and all irrigation treatments.

attributed to differences in water retention characteristics of the profiles. The soil at location 2 was deep and well-drained, whereas the other two were shallow, stratified soils in which water is retained at low tensions following irrigation. Thus surface evaporation, with accompanying accumulation of nitrate-nitrogen at the soil surface, persisted for a considerable time following irrigation on the stratified soils. This surface isolation was probably appreciable on these soils for the shallow placement whereas such accumulations were negligible on the well-drained soil.

Effects on sugar content

Results of sugar content determinations are presented in Figure 4. In view of the placement effects noted previously and the probability of confounding by these effects, only data on the plowed-down applications for Moses Lake location 1 and the Toppenish location are presented.

Sugar contents were significantly reduced, as expected, at increasing nitrogen rates. Pronounced irrigation effects were apparent at the two Moses Lake locations. Higher sugar contents resulted from excess water applications, especially at the high nitrogen rates, indicating a reduced plant nitrogen level in the over-irrigated treatments. Failure to obtain such behavior at the Toppenish location is probably due to the high indigenous nitrogen level and the earlier harvesting.

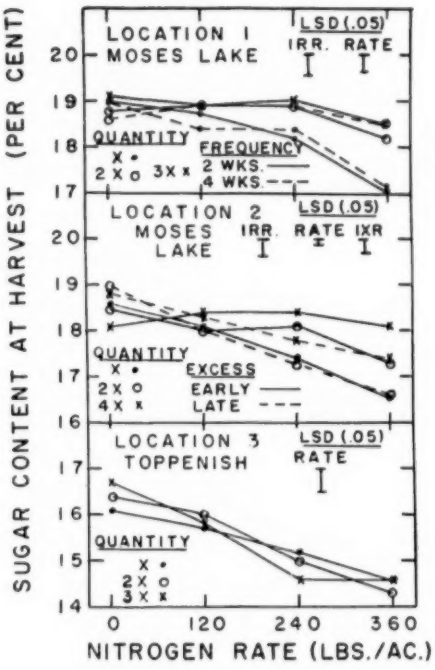


Figure 4.—Effects of nitrogen rate and irrigation on sugar content of beets at harvest for three locations, 1954.

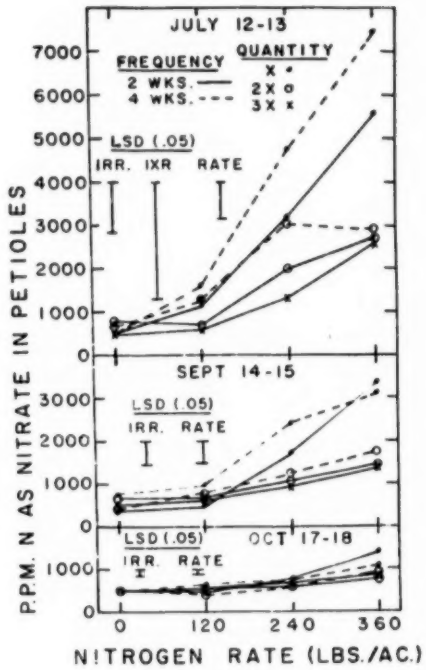


Figure 5.—Effects of nitrogen rate and irrigation on nitrate-nitrogen level of beet petioles from the plowed down placement at location 1, 1954.

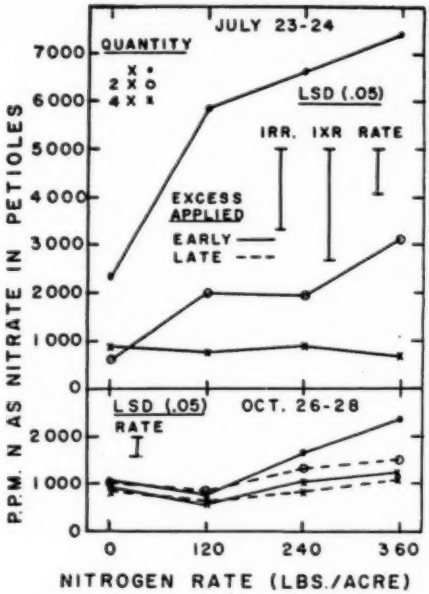


Fig. 6.—Effects of nitrogen rate and irrigation on nitrate-nitrogen level in beet petioles from the plowed-down placement at location 2, 1954.

Effects on plant nitrogen level

Results of nitrate-nitrogen determinations for the plowed-down nitrogen placement at locations 1 and 2 are presented in Figures 5 and 6.

Marked reductions in nitrate concentration resulted from the excess water applications. The effects of the excess applications early in the season, shown in Figure 6, again emphasize that the early irrigations are most critical with regard to nitrogen loss by leaching.

At location 1, the nitrate concentration was higher in the petiole samples from the plots irrigated at the four-week frequency than from the plots irrigated at two-week intervals. However, this condition cannot be assumed to hold true for the entire irrigation cycle for the two frequencies. The nitrate concentration in the plants irrigated at 4-week intervals may, in fact, drop considerably below that of those irrigated every two weeks, since the samples were taken about seven to ten days following irrigation of all treatments. Further, the nitrate levels determined do not accurately portray the total nitrogen uptake by the treatments.

However, the fact that the nitrate levels were well above the critical level on the four-week frequency indicates that the yield differences obtained between frequencies was not due to a nitrogen-irrigation interaction but were probably real moisture responses.

Nitrate determinations for the disced-in applications at location 1 showed the nitrate level to be as high or higher than that of the plowed-

down applications at the July 12-13 sampling. However, it is impossible to determine the total nitrogen uptake for these treatments from the material available. Thus, the yield differences between placement methods cannot be definitely explained from the present data.

Summary

1. Application of irrigation water in excess of that storable in the root zone early in the growing season materially reduced sugar beet yields. Similar late-season applications did not affect yield. Yield reductions were considerably greater at low nitrogen rates than at high rates.

2. Lower yields were obtained on shallow, stratified soils where applied nitrogen was disced-in than where the nitrogen was plowed-down or side-dressed. On a deep, well-drained soil, placement effects were negligible. The reductions obtained on the stratified soils are believed to result from surface isolation of nitrogen by water moving upward and evaporating at the soil surface.

3. Sugar content at harvest was considerably higher on treatments receiving early excess water applications than on the control irrigation treatments. The differences were greatest at high nitrogen rates. Late-season excess water applications increased the sugar content to some degree, also. This further indicates a reduced nitrogen status of the plants prior to harvest, and that even late-season excess applications may remove sufficient nitrogen to affect sugar content.

4. Nitrate-nitrogen levels in sugar beet petioles was greatly reduced by early excess water applications and, to a lesser degree, by similar applications late in the season. In 1953, excess applications appeared to increase the availability of shallow sidedressed nitrogen. No conclusive evidence was obtained in 1954 relative to the effect of irrigation practice on availability of nitrogen applied by different methods.

References

(1) PRUITT, W. O. and JENSEN, M. C. 1955. Determining when to irrigate. Agric. Eng. 36:6, 389-393.

(2) ROBINS, J. S. 1954. Irrigation and nitrogen fertilization experiments on sugar beets in the Columbia Basin. Wash. Agr. Expt. Sta. Circ. 249.

(3) ROBINS, J. S., NELSON, C. E., and DOMINGO, C. E. 1956. 1954 irrigation and nitrogen fertilization experiments on sugar beets in the Columbia Basin. Wash. Agr. Expt. Sta. Circ. 278.

(4) ULRICH, ALBERT. 1948. Plant analysis as a guide to the nutrition of sugar beets in California. Proc. Amer. Soc. Sugar Beet Tech. pp. 364-377.

Studies on the Control of Saponin Concentration in Refined Beet Sugar

ROBERT S. GADDIE[1]

All commercial granulated sugars contain traces of acid-insoluble substances which precipitate in acidified aqueous solution and which, at sufficient concentration, show up as a white flocculent precipitate. The floc present in beet sugar is composed of a mixture of saponin and its derivatives, of fats, and of various other adsorbed minerals and colloids. (1)[2] Because these substances gradually precipitate in certain carbonated beverages and acidified pharmaceutical syrups, it is necessary that sugar be produced as free as possible from these floc-formers.

Certain information of a very general nature on the concentration of floc in the granulated sugars produced by the seven factories of the Utah-Idaho Sugar Company was obtained during the 1952 and 1953 campaigns. The general nature of the information was due in large part to the extreme qualitative value of the then known tests. In 1953, Walker and Owens, working on this problem, found a fairly large percentage of the acid-insoluble constituents in beet sugar to be saponin and Dr. Walker devised a method of analysis for this component. Even though the method had not been published and had, in fact, not been tested thoroughly, Walker very kindly made available all the information he had on the problem. With our adaptation of this test it was determined that a measure of saponin concentration in a sugar gave an excellent measure of that sugar's tendency to produce floc in susceptible beverages. The maximum safe concentration of four p.p.m. for sugar going into these susceptible beverages (2) was further determined. With these facts established, it was decided to make exhaustive tests on sugar from all seven of the Utah-Idaho factories during the 1954 campaign and attempt, within the practical limits of full scale operation and on present knowledge, to control the saponin concentration in the final sugar.

The number of analyses which could be made was limited by the two spectrophotometers available. However, a minimum of three samples, representing three to six strikes of sugar from each factory each day, was analyzed. A study of these analytical results showed that each factory's sugar production fell into one, and only one, of three classifications. Typical examples of these three are shown in Table 1.

Table 1.—Saponin Concentration in Sugar Samples from Three Different Factories.

Factory	No. of Samples	Saponin Concentration ppm		
		Maximum	Minimum	Average
A	232	24	6	14
B	298	3	0	1
C	631	17	1	6

[1] General Chemist, Utah-Idaho Sugar Co., Salt Lake City, Utah.
[2] Numbers in parentheses refer to literature cited.

The results gave quantitative confirmation to deductions arrived at from the general information obtained the two previous years.

a. Sugar from some factories always contains relatively high concentrations of saponin, (A in Table 1).

b. Sugar from some factories always contains very low concentrations of saponin, (B in Table 1).

c. Saponin concentration in sugar from some factories varies within wide limits (C in Table 1).

Because the saponins are only one of many non-sucrose constituents in purified beet juice and because very efficient sugar-end operation nearly always results in improved sugar quality, it is logical to suppose that saponin concentration in granulated sugar would be lowered by high pan purities, precision sugar boiling technique to avoid crystal agglomerates and as near perfect separation of mother liquor from crystals as possible. On this supposition it was decided to try this procedure at Factory C (Table 1). Standard liquor purities were held up to near 93.0, high raw sugar purity was maintained at 98.5 to 99.0 and sufficient wash water was applied at the centrifugals to bring the color index of the sugar to the lowest practical value. Typical results are shown in Table 2. Colors are given in terms of reference basis units (3).

Table 2.—Saponin Concentration in Selected Sugar Samples from Factory C.

Sample No.	White Pan Purity	Color Index	Saponin p.p.m.
1	92.5	34	12
2	92.8	29	8
3	92.1	34	11
4	92.6	34	10
5	92.9	27	6
6	91.9	22	3
7	93.1	17	3
8	95.1	13	1
9	92.5	17	1
10	92.2	19	2
11	93.2	19	1

Variations in pan purity within the limits at which operations were conducted did not have any measurable effect on floc, but floc concentration at this factory was found to be almost directly proportional to color index. However, in order to obtain a sugar with a color index below 22, it was sometimes necessary to wash with as much as 26 quarts of water per machine. This would fill up the white side in about 24 hours and so would have to be discontinued temporarily. Throughout most of the campaign, however, it was possible to obtain sugar containing less than four p.p.m. saponin by washing to such a color index.

As soon as results from the work at factory C were available, identical conditions were established at factory A where saponin concentration had been running consistantly high. All operating conditions were directed toward the production of an extremely even, relatively coarse grain in the

vacuum pans, and as near perfect washing in the white centrifugals as could be obtained. In spite of these efforts, results obtained at Factory C could not be duplicated in Factory A, even for short periods. There seemed to be no correlation whatever between saponin concentration and any other physical or chemical characteristic of the sugar. This is illustrated in Table 3.

Table 3.—Saponin Concentration in Selected Sugar Samples from Factory A.

Sample No.	White Pan Purity	Percent Ash	Color Index	Saponin p.p.m.
1	93.1	.0050	17	12
2	93.8	.0054	20	8
3	93.6	.0058	23	13
4	93.4	.0038	24	15
5	93.3	.0040	35	8
6	93.5	.0044	35	10
7	93.5	.0046	36	6

Periodic samples of sugar were made into a massecuite, purged, and washed in the laboratory centrifugal with what would be equivalent in factory practice to 40 quarts of hot distilled water per machine without effecting a decrease of more than 1 or 2 p.p.m. of saponin.

Since the saponins are highly surface-active, adsorbing agents such as activated carbon should remove at least a portion of those left in the purified juice (4). Therefore, the addition of activated carbon (Darco S51) to the standard liquor was started. The first 48 hours, .3 pounds per ton of beets was added. For the next 48 hours this was increased to .5 pounds and 48 hours later to .6 pounds per ton of beets. At this point, the standard liquor filters had about reached the limit of their capacity and higher carbon additions were not practical. During these periods, sugar boiling was carefully watched and 25 quarts of centrifugal wash water per machine were used. No decrease in average saponin concentration in the sugar was effected by any of these carbon additions. Adsorbing agents, such as activated carbon, have selective adsorption properties which do not confine themselves to the saponins. Because of this, Sabine predicted, and our work has shown, that in many cases the quantity of carbon which would probably be required would raise production costs higher than could be justified.

Saponins all form emulsions with oil (6). In testing the solubility of the sugar beet saponins in the various common defoamers used in sugar manufacturing, McGinnis (7), found that they were least soluble in Balab. Therefore, for a period of 24 hours, all foam oils (except Balab) were taken out of the process and Balab used as sparingly as possible. Saponin concentration in the sugar produced during this period ranged from 6 to 13 p.p.m.

Triterpenes form insoluble compounds with heavy metal ions (4). This explains the high (about 95%) elimination from diffusion juice, as the calcium complex, when lime is added at the carbonators. Hoping to take further advantage of this property, two things were tried. First, milk of lime equivalent to about .15% CaO on beets was added to the filtered 1st carbonator juice going to 2nd carbonation. This was continued for 3 days

and when no decrease in floc was obtained the second experiment was started. This consisted of adding fresh milk of lime equivalent to approximately .07% CaO on beets to the standard liquor at the high melter. This was then sulfured to pH 7-8 to 8.0 using liquid SO_2 and filtered on the standard liquor presses using .6 pounds filteraid per ton of beets. After a 72 hour run in which no significant decrease was effected in the average saponin concentration in the sugar, this procedure was also discontinued.

It is highly probable that methods of processing influence, to some extent, the concentration of saponins in the final sugar. Eis, et al (4) have shown, that even at the same factory, the concentration of triterpenes in diffusion juice is widely variable. Although 95-97% is eliminated in carbonation, in cases of high triterpene incidence, the removal is still inadequate.

References

(1) WALKER, HOWARD G. JR., and OWENS, HARRY S. 1953. Beet sugars; acid insoluble constituents in selected samples. Agr. and Food Chem. 1:450.

(2) WEST, ROBERT R. and GADDIE, ROBERT S. 1956. Determination of saponin in granulated sugar: method of H. G. Walker adapted to routine control. Journ. Amer. Soc. Sugar Beet Tech. 9:146-152.

(3) Bottler's Sugar Survey (1952)

(4) EIS, F. G., CLARK, L. W., MCGINNIS, R. A., and ALSTON, P. W. 1952. Floc in carbonated beverages. Ind. & Eng. Ch. 44:2844.

(5) SABINE, F. M. 1952. The floc problem in beet sugar. Proc. Amer. Soc. Sugar Beet Tech. pp. 760-763.

(6) MCILROY, R. J. 1951. The plant glycosides. Edward Arnold & Company, London. p. 64.

(7) MCGINNIS, R. A. Private communication.